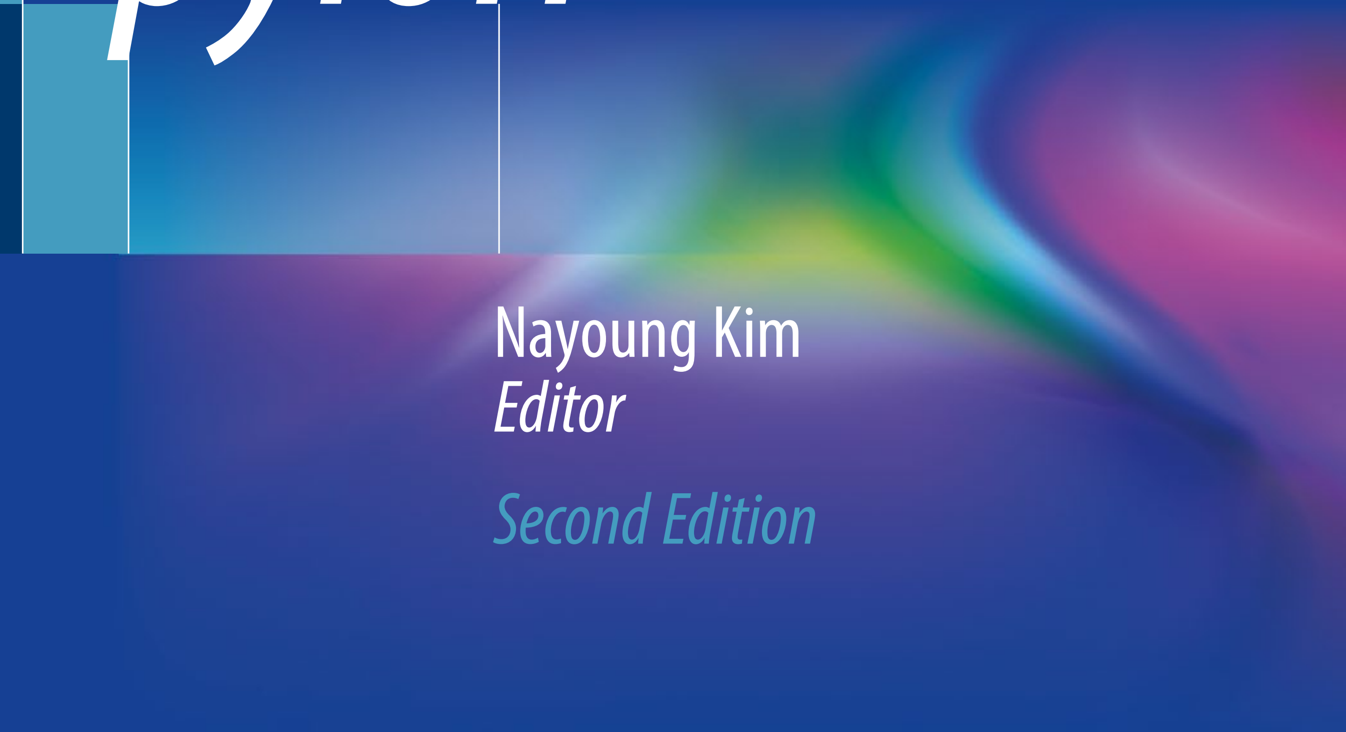
Helicobacter pylori



Nayoung Kim
Editor

Second Edition

Springer

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Preface

I am really happy to revise the *Helicobacter pylori* which has been published in 2016 by Springer Co. It was good news that there has been steady downloading of the book chapters by medical students; trainees such as interns, residents, and fellows; and boards who have interests in this area in the world. In 2016, experts including me expected that many issues would be resolved in the clinical areas soon. However, there have been steady publications of new articles in the various aspects of *Helicobacter pylori* (*H. pylori*) in the world and new knowledge have been accumulated for 7 years. Furthermore, the literature raised new issues which needs more research for the clinical application in each age and sex. It means that precision medicine is also needed in the various fields of *Helicobacter pylori*-induced gastroenterological diseases. In this second edition, all the chapters have been updated based on the many publications including meta-analysis and systemic review. Actually, gastric cancer is still the leading cause of cancer incidence and mortality in the world. It means we still need the primary and secondary prevention strategy to prevent primary gastric cancer in addition to metachronous gastric cancer. In addition, the new infection of *H. pylori* continues, especially, in the underdeveloped countries although it is continuously decreasing in South Korea, Japan, and China where the gastric cancer is still high in the world. Thus, we need recent epidemiologic data not only for adults but also children from the entire world to expect the trend of *H. pylori*-induced gastroduodenal diseases. Especially, there have been new knowledge regarding the interaction of *H. pylori* and non-*H. pylori* microbiota in the gastric habitat in terms of gastroduodenal diseases such as gastric cancer and premalignant diseases such as atrophic gastritis and intestinal metaplasia due to the wide use of next-generation sequencing and sometimes shotgun sequencing. Active interaction of *H. pylori* and mucosal immunology causes intragastric diseases and gives impact on the extraintestinal diseases such as endocrine, cardiovascular, hematologic, neurologic, autoimmune diseases, and lower gastrointestinal disorders such as colon adenoma and colon cancer. Many of these diseases are related with the systemic effects of CagA toxin and cytokines. So far, many papers regarding the long-term effects of *H. pylori* eradication on these extraintestinal diseases were published which is further extended from the association of *H. pylori* and the extraintestinal diseases. Consequently, new chapters were added in the second edition *Helicobacter pylori* book. That is, diagnosis of *H. pylori* infection after gastric surgery, and extraintestinal manifestations of *H. pylori* infection: lower gastrointestinal disorders and neurologic disease. In addition, as extraintestinal manifestations are different depending on sex and age the chapter entitled metabolic parameters and glycosylated hemoglobin A1C has been added. Furthermore, as the long-term change of non-*H. pylori* microbiota after *H. pylori* eradication has been uncovered, the new chapter entitled the effect of *H. pylori* eradication on the gastric microbiota was newly described. The guideline for *H. pylori* eradication became more expanded because it became clear that its eradication brings more benefit for humans. However, the increase of antibiotic resistance is still the most important barrier for this strategy. Thus, in the second edition *Helicobacter pylori* many trials of *H. pylori* eradication methods and appropriate approaches for its effective eradication are updated. I can say that this book has been successfully updated regarding all of the important issues of *H. pylori* from the basic area such as how it survives in the acid gastric mucosa, immunology, and pathophysiology to the clinical aspect such as epidemiology, diagnosis, symptom, disease, antibiotic resistance, treatment, consequences of *H. pylori* eradications, and animal model up to the early 2023.

I would like to thank the 26 Korean doctors for their contribution to the *Helicobacter pylori* book. In addition, I would like to express my sincere appreciation to Prof. David Y. Graham, Francis Megraud, Ernst J. Kuipers, and Elizabeth A. Marcus for the submission of updated manuscripts. They are the leading experts in the world trying to resolve many issues in each area. I am very grateful to Ms. Jihyun Park who edited the contents and references of all the chapters. Without her help, this book could not be completely revised.

In conclusion, I am very pleased to publish this second edition by Springer.

Seongnam, Gyeonggi-do, South Korea Nayoung Kim

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Part I

Epidemiology

1

Prevalence and Transmission Routes of *H. pylori*

Nayoung Kim

Abstract

To establish health policies for the prevention of *Helicobacter pylori* (*H. pylori*)-related diseases, observation of prevalence trends and confirmation of risk factors for *H. pylori* infection are important. New infections of *H. pylori* have been falling due to improved sanitation and better living conditions over the decades. However, its prevalence has been reported to be still higher than 50% in 2013 in Africa, Central/South America, some areas of Asia, and South/East Europe and at least twofold higher in countries with high gastric cancer (GC) incidence. In South Korea where the incidence of GC is still the highest in the world, the prevalence of *H. pylori* decreased to 41.5% in 2016–2017. In contrast, in Northern Europe and North America, about one-third of adults are infected. However, even in these countries, *H. pylori* remains highly prevalent among the immigrants coming from countries with a high prevalence of *H. pylori*. The lower prevalence of infection in children aged around 5–10% even in the presence of high prevalence in adults suggests a further decline of *H. pylori* prevalence in the coming decades. Low socioeconomic conditions in childhood are the most important risk factors for *H. pylori* infection. Although the way of transmission is still unclear, interpersonal transmission appears to be the main route, especially, in the developed countries.

Keywords

Helicobacter pylori · Prevalence
Transmission · Epidemiology

1.1 Introduction

The prevalence of *Helicobacter pylori* (*H. pylori*), a cause of peptic ulcer disease, gastric adenocarcinoma, and low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma [1], has been falling due to improved sanitation and better living conditions over the decades in most countries [2–4]. The changing epidemiology of the bacterium has been associated with a decline in the peptic ulcer disease and gastric cancer (GC) [5] but it could increase gastroesophageal reflux disease and asthma, which are related to acid or immunity [6, 7]. There are many studies regarding the prevalence and risk factors of *H. pylori* infection, and older age was commonly considered as the main risk factor [4, 8, 9]. Adults have a continuous risk of *H. pylori* infection, resulting in increased seroprevalence during

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lifetime as a function of age [10]. However, this does not mean that young people have a higher seroprevalence when they get older, as cross-sectional presentation does not necessarily give an accurate picture of lifetime trends. To compensate for this, there have been precious studies on lifetime trends for *H. pylori* seroprevalence [8, 9, 11, 12]. In this chapter, the epidemiology and risk factors of *H. pylori* will be summarized over the decades depending on adults and children. In addition, the transmission route will be presented according to developed and developing countries.

1.2 Prevalence of *H. pylori*

This chapter has received much help from two review articles, which have been published in 2014 [13, 14]. Eusebi et al. [14] have searched PubMed up to September 2013, and Peleteiro et al. [13] made a search from Medline and PubMed databases for the period of April 2013–March 2014, in which results have been well introduced in this review. In addition, I tried to update the prevalence of *H. pylori*, which has been published in PubMed until now. The characteristics of the populations and tests in the publications were rather variable. That is, the clinical setting such as socioeconomic status was different. The assessment of *H. pylori* status was done mainly through enzyme-linked immunosorbent assay (ELISA) tests to determine IgG antibody titers in serum, but few studies used urine, saliva, or urea breath test (UBT) and joint information of blood and biopsy specimens. Thus, the results of the prevalence rate of *H. pylori* in one country in a similar period could be at a wide range sometimes. As age is a very critical factor for the prevalence of *H. pylori*, the prevalence results are shown separately for adults and children in this chapter.

1.2.1 Prevalence of *H. pylori* in the Adults

The prevalence of *H. pylori* increased overall with age, decreasing in the older age groups such as those over 60 or 70 years old in some countries (Chile [15], Ecuador [16], Japan [17], Mexico [18], Latvia [19], and South Korea [9, 20]). In the late 1990s/early 2010s, the prevalence estimates were generally higher among countries in Central/South America and Asia [13], which decreased in the late 2010s in most of these countries.

1.2.1.1 Asia Pacific Area

As the GC incidence is highest in Korea, Japan, and China, the trend of prevalence of *H. pylori* in these countries was compared. All of these countries showed a decrease in the prevalence of *H. pylori*, but the details were slightly different. That is, the prevalence of *H. pylori* was 71.4% in China (35–64 years, 1989) [21], which decreased in 2000 (Table 1.1). That is, a total of 5417 healthy individuals aged between 30 and 69 years from areas of high incidence of GC in China were tested with ^{13}C-UBT, and the prevalence of *H. pylori* infection was 63.4% in the 2014 report [30]. When two Chinese prevalence studies using UBT or serum IgG antibody were compared by age, the 2004–2005 Jiangsu study [28] showed a higher rate than the 2003 Beijing study [29] regardless of age. Another study included 51,299 subjects aged ≥18 years who underwent health checkups between April 2013 and June 2016 in urban city of China using *H. pylori* urease-IgG antibodies [31].The overall *H. pylori* prevalence was found to be 31.9%, with the highest prevalence in the 1950–1959 birth cohort [31] (Fig. 1.1a). Similarly, Japan showed an *H. pylori* prevalence rate of 70.0% among 40–79 years old in 1988–1990 [53] and 75.0% among 40–69 years old in 1990–1992 [54]. However, three subsequent Japanese prevalence studies of *H. pylori* (using urine antibody or serum IgG antibody) in 1992 [83], 2002–2006 [17], and 2007–2011 [84] showed a definite decrease in prevalence rate as time regardless of age (Fig. 1.1c). In South Korea, the decrease of seroprevalence was significant across all age groups and in most areas of the country, especially in the age below 40 years old (Fig. 1.1b), which reflects the changes from developing to developed country. That is, in a large cross-sectional nationwide multicenter

Table 1.1 Prevalence of *H. pylori* infection reported in adults

Country	Year reference	Setting	Number	Diagnostic method	Prevalence of *H. pylori* (%)
Argentina	2000 [22]	B	754 (261 children and 493 adults)	Serum IgG	Crude 37.5/ Adjusted 35.7
Australia	2008 [23]	B	2413	Serum IgG (ELISA)	15.4
	2011 [24]	A	1400	Serum IgG (ELISA)	15.5
Bhutan	2013 [25]	A	372	Histology and RUT	73.4
	2014 [26]	A	244	Serum IgG (ELISA)	86
Canada	2013 [27]	A	203	Gastric biopsy tested positive for the bacterium	37.9
Chile	2007 [15]	A	3619	Serum IgG (ELISA)	Crude 73.0/ Adjusted 73.4
China	2008 [21]	A	8280	*H. pylori* antibody in urine (ELISA)	71.4
	2008 [28]	A	1371	^{13}C expired air (UBT) and Serum IgG (ELISA)	62
	2009 [29]	A	1232	^{13}C expired air (UBT)	41.3–54.7
	2014 [30]	A	5417	^{13}C expired air (UBT)	63.4
	2017 [31]	A	51,299	*H. pylori* urease IgG	31.9
Cyprus	2013 [32]	A	103	Gastric biopsy, PCR (primers for urea)	39.8
Czech Republic	2006 [33]	B	2509 (1234 men and 1275 women)	^{13}C expired air (UBT)	41.7
	2012 [34]	B	1837 (857 men and 969 women)	^{13}C expired air (UBT)	23.5
Ethiopia	2013 [35]	A	1388	Anti-*H. pylori* antibodies of all isotypes (IgG, IgM, IgA)	65.7
Finland	1999 [36]	A	173	Serum IgG (EIA)	61.0
	2001 [37]	A	730 (1983)	Serum IgG (EIA)	30.1 (1983)
			618 (1995)		13.1 (1995)
	2006 [38]	A	336	Serum IgG (not further specified)	65.0 (1977–1980)
					59.0 (1997–1998)
	2009 [39]	A	958	Serum IgG (EIA)	31.0 (1983)
					21.0 (1989)
					24.0 (1995)
					19.0 (2001)
France	1999 [40]	A	1597	IgG in saliva (ELISA)	25.4
Germany	1999 [41]	A	1834	Serum IgG (ELISA)	39.3
	2005 [42]	A	6545	Serum IgG (ELISA)	40.7
Iceland	2005 [43]	A	96 (only women)	Serum IgG (ELISA)	33.0
India	2013 [44]	A	530	Histology and RUT	58
	2013 [45]	A	530	Histology and RUT	62
Indonesia	2015 [46]	A	267	Culture, histology, and RUT	22.1
	2016 [47]	A	193 (47 men and 146 women)	^{13}C-UBT	15
Iran	2014 [48]	B	8459 (3575 men and 4172 women)	Serum IgG (ELISA) and stool antigen	30.6–82
	2016 [49]	A	Meta-analysis	Meta-analysis (heterogenous)	62
Israel	2000 [50]	A	144	Serum IgG (ELISA)	46.5

(continued)

Table 1.1 (continued)

Country	Year reference	Setting	Number	Diagnostic method	Prevalence of *H. pylori* (%)
Italy (Sardinia)	2015 [51]	A	11,202 (4160 men and 7042 women)	Histology, RUT, or ^{13}C expired air (UBT)	43.8
Japan	2005 [52]	A	350	Serology	19.7
	2005 [53]	A	633 (349 men and 284 women)	*H. pylori* antibody in serum (not further specified)	70.0
	2006 [54]	A	511 (342 men and 169 women)	Serum IgG (ELISA)	75.0
	2007 [55]	B	4136	Serum IgG (ELISA)	54.7–67.5
Kazakhstan	2013 [56]	A	835	Serum IgG (ELISA)	76.5
Latvia	2012 [19]	A	3564 (1218 men and 2346 women)	Serum IgG (ELISA)	79.2
Lebanon	2012 [57]	A	308 (144 men and 164 women)	Serum IgG (ELISA)	52.0
Malaysia	2016 [58]	A	275 (202 men and 153 women)	Serum IgG (ELISA)	44.7
Mexico	1998 [18]	B	11,605	Serum IgG (ELISA)	66.0
	2013 [59]	A	343	Serum IgG (ELISA)	52.2
Morocco	2013 [60]	A	429	Histology, RUT, culture	75.5
New Zealand	2014 [23]	11–85 (A)	4463	Serology	30.2
Nigeria	2013 [61]	A	125	Serum IgG (ELISA) / histology	93.6/80.0
Oman	2013 [62]	A	133 (100 men and 33 women)	Serum IgM, IgG, and IgA (ELISA)	69.5
Portugal	2013 [63]	A	2067	Serum IgG (ELISA)	84.2
Republic of San Marino	1995 [64]	A	2237 (1048 men and 1189 women)	Serum IgG (ELISA)	51.0
Saudi Arabia	2013 [65]	A	456	Serum IgG (ELISA)	28.3
Singapore	2002 [66]	A	261 (130 men and 131 women)	Serum IgG (ELISA)	50.2
South Korea	2001 [67]	A	3394	Serum IgG (ELISA)	66.9
	2005 [68]	A	344 (228 men and 116 women)	Serum IgG (ELISA)	80.8
	2007 [20]	A	15,916 (8616 men and 7300 women)	Serum IgG (ELISA)	56.0
	2008 [69]	A	25,536	Serum IgG (not further specified)	59.2
				Urease enzyme in biopsies (RUT)	
				H. pylori presence in histological examination	
	2013 [8]	A	10,796 (6085 men and 4711 women)	Serum IgG (ELISA and EIA)	54.4
	2018 [9]	A	16,885 (8950 men and 7935 women)	Serum IgG (ELISA and EIA)	43.9
Sweden	2003 [70]	A	3502	Serum IgG (ELISA)	18.0
	2004 [71]	A	499 (414 men and 85 women)	Serum IgG (ELISA)	40.0
	2011 [72]	A	117	Serum IgG (ELISA)	35.0
	2016 [73]	A	388 (186 men and 202 women)	EIA	15.8

Table 1.1 (continued)

Country	Year reference	Setting	Number	Diagnostic method	Prevalence of *H. pylori* (%)
The Netherlands	2013 [74]	A	1550	Serum, *H. pylori* antibody, and CagA antigen	31.7
	2013 [75]	A	6837	Serum IgG and CagA antibodies	46
Taiwan	2003 [76]	B	924	Serum IgG (ELISA)	16.7
Turkey	2003 [77]	A	675/260/148[a]	Serum IgG (ELISA)	13.1/30.4/44.5
	2013 [78]	A	4622	^{13}C expired air (UBT)	82.5
The United Kingdom	2002 [79]	B	10,118	Serum IgG (ELISA)	13.4
The United States	2000 [80]	A	7465 (3717 men and 3748 women)	Serum IgG (ELISA)	32.5
	2005 [81]	B	7462	Serum IgG (ELISA)	27.1
	2015 [82]	A	1200	Culture or histopathology	28.9

A adults, *B* both children and adults, *EIA* enzyme immunoassay, *ELISA* enzyme-linked immunosorbent assay, *RUT* rapid urease test, *PCR* polymerase chain reaction, *UBT* urea breath test

[a] Three subgroups were recruited encompassing 675 Germans (402 men, 273 women), 260 Turkish people born and raised in Germany (145 men, 115 women), and 148 Turkish people living in Turkey (91 men, 57 women)

study, more than 10,796 asymptomatic subjects without a history of *H. pylori* eradication were enrolled in the adult age group greater than and equal to 16 years in 2011; its prevalence was 54.4% [8]. In addition, it further decreased to 43.9% among 16,885 asymptomatic subjects without a history of *H. pylori* eradication in the adult age group greater than and equal to 16 years in 2016–2017 [9] (Fig. 1.1b). This result was significantly lower than that reported in the same country by similar surveys performed in 1993–1999 [68], 1998 [67], 2005 [20], and 2006 [69] where the prevalence of *H. pylori* was 80.8% [68], 66.9% [67], 59.6% [20], 59.2% [69], and 54.4% [8] respectively (Table 1.1), in the age of greater than and equal to 16 years [8]. This decreasing trend could be explained by cohort analysis [8] instead of continuous new infections over the age. All younger birth cohorts had a lower seroprevalence of *H. pylori* than older birth cohorts at the same age, and a decreased seroprevalence within the same birth cohorts was also accounted for in this phenomenon [8] (Fig. 1.2). The significant difference of *H. pylori* prevalence between male and female was the same until 2016–2017 [9]. The prevalence in Singapore was already not so high in 1998, at 50.2% (55–69 years) [66] (Table 1.1). In India, the prevalence of infection ranged from 58 to 62% in subjects with dyspeptic symptoms [44, 45], and in Kazakhstan, the prevalence of *H. pylori* was 76.5% among symptomatic and asymptomatic cases [56]. Similarly, in Bhutan, the infection was present in 73.4% of cases, although it was lower in the capital city, Thimphu, than in the rural areas, mainly related to sanitary conditions [25]. An even higher prevalence rate of 86% was reported from another study in the same country [26] (Table 1.1). In contrast, *H. pylori* prevalence in Indonesia is relatively low. One study showed that, in 193 subjects from a low-income community in Northern Jakarta, it was only 15% [47]. Similarly, another Indonesian study showed that in the 267 patients in Java, Papua, Sulawesi, Borneo, and Sumatra Islands, the overall prevalence was 22.1% [46] (Table 1.1). Orang Asli tribes in peninsula Malaysia *H. pylori* prevalence was 44.7% (115/275) [58] (Table 1.1).

In Oman, one of the Eastern Mediterranean regions, *H. pylori* prevalence was reported to be 69.5% among 15- to 50-year-olds by serology [62]. Similarly, in a population-based cross-sectional survey in Turkey, a weighted overall prevalence of infection was 82.5% among more than 4600 subjects [78] (Table 1.1). Interestingly, the prevalence was lowest among individuals liv-

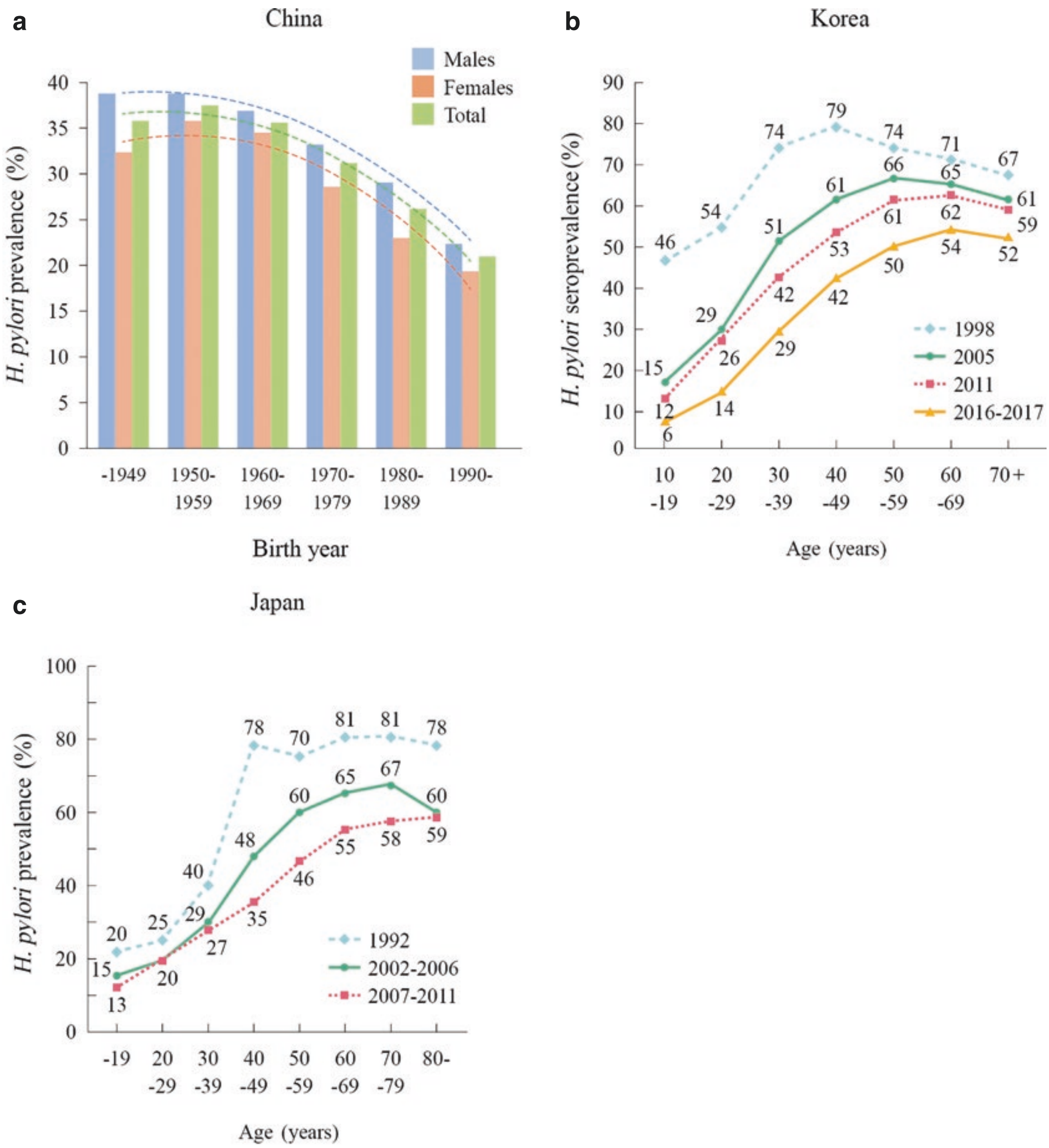

Fig. 1.1 Comparison of prevalence rate of *H. pylori* infection among China, Korea, and Japan. (**a**) Birth year and the *H. pylori* prevalence in China. Males: $\chi^2 = 222.232$, *p*-value for trends = 0.000; females: $\chi^2 = 266.960$, *p*-value for trends = 0.000; total: $\chi^2 = 482.885$, *p*-value for trends = 0.000 (Adapted from Yu et al. [31]) (**b**) Seroprevalence in asymptomatic subjects without a history of *H. pylori* eradication in 1998 [67], 2005 [20], 2011 [8], and 2016–2017 [9] in Korea. (**c**) Prevalence (using urine antibody or serum IgG antibody) of *H. pylori* in 1992 [83], 2002–2006 [17], and 2007–2011 in Japan [84]

ing in the southern part of Turkey who usually have a citrus fruit-rich diet, as this is the major citrus fruit-growing area [78]. In the Eshraghian review article regarding *H. pylori* prevalence in ten studies from Iran, the overall prevalence of *H. pylori* infection ranged from 30.6 to 82% irrespective of time and age group [48]. In 2016, there was a meta-analysis using 21 studies, and the *H. pylori* prevalence was 62%. In addition, the prevalence of *H. pylori* infection from the Eastern Mediterranean region (five studies from the Kingdom of Saudi Arabia, four studies from Egypt, two studies from the United Arab Emirates, and one study from Libya, Tunisia, and

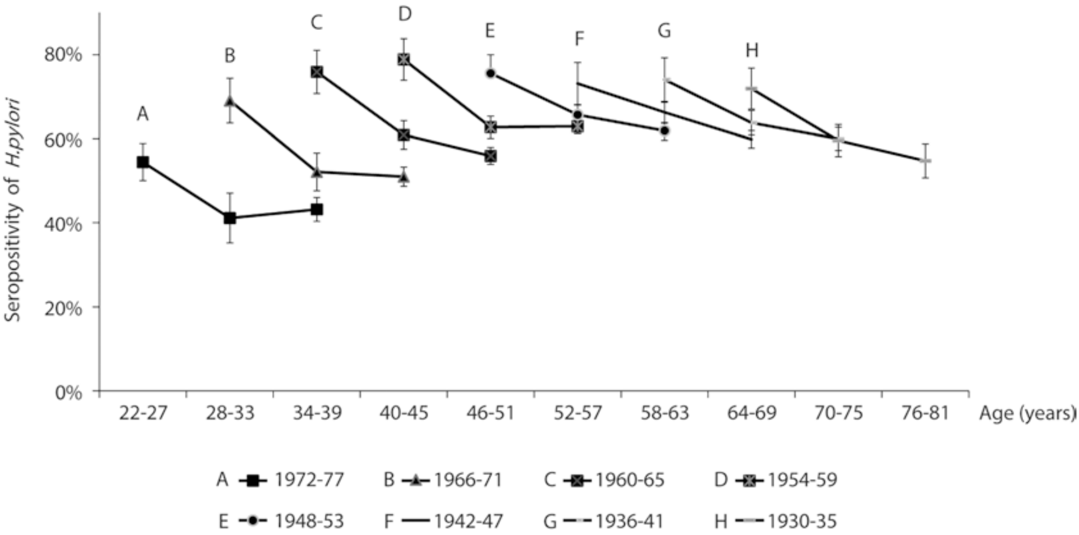

Fig. 1.2 Seroprevalence of *H. pylori* infection in asymptomatic subjects without a history of *H. pylori* eradication in birth cohort by age in Korea. Each line connects the values for the same cohort group in different age groups. For example, the first line shows the seroprevalence of *H. pylori* in a birth cohort in 1972–1977 for ages 22–39 years, and the second line shows the seroprevalence of *H. pylori* in a birth cohort in 1966–1971 for ages 28–45 years. All younger birth cohorts at the same age have a lower seroprevalence of *H. pylori* than older birth cohorts (Adapted from Lim et al. [8])

Lebanon) ranged from 22 to 87.6% [48] (Table 1.1). In the case of Israel, the prevalence rate was 46.5% (mean age 18.7 years, 1986–1995) [50]. This wide variation in Middle Asia is probably related to the various age groups and methodology of *H. pylori* tests.

In Australia, two seroprevalence rate studies performed in 2002 for 1–59 years old [23] and during 2002–2005 [24] showed 15.4% and 15.5%, respectively (Table 1.1). In the case of New Zealand where several ethnic groups live, *H. pylori* prevalence was highest among Pacific people (ranging from 39 to 83%) followed by Maori (18–57%) and then European (7–35%) [85] (Table 1.1). The absolute ethnic differences in seroprevalence are decreased in subsequent cohorts, but the relative ethnic differences increased [85].

1.2.1.2 Europe

In Europe, the prevalence of *H. pylori* seems to be lower in Northern countries than in Southern and Eastern countries [14]. In the Netherlands, a randomly selected sample of 1550 blood donors from four different regions was tested for the presence of antibodies against *H. pylori* and the CagA antigen [74]. This study where only native Dutch subjects were evaluated excluding non-European immigrants reported a 31.7% prevalence of *H. pylori* infection (Table 1.1), with 28% of *H. pylori*-positive subjects carrying a CagA-positive strain [74]. The seroprevalence of *H. pylori* declined from 48% in subjects born between 1935 and 1946 to 16% in those born between 1977 and 1987, as a likely consequence of a birth cohort effect. Moreover, the proportion of CagA-positive subjects decreased from 38% to 14% in the same age cohorts. Additionally, from the Netherlands, a population-based prospective study of a cohort of more than 6500 pregnant women was published [75]. This study found that the prevalence of *H. pylori* in Dutch women was 24%. In contrast, the prevalence of *H. pylori* in non-Dutch women was much higher, 64% [75]. Moreover, in the latter group, infected subjects born abroad (first-generation immigrants) had a higher risk of *H. pylori* infection than second-generation immigrants [75].

In Sweden, the prevalence of *H. pylori* was 18.0% (17–79 years, 1995) [70], 40.0% (51–79 years, 1995–1997) [71], and 35.0% (16–40 years, 1968–2001) [72]. In addition, the prevalence of positive *H. pylori* serology (EIA) decreased from 37.9% (1989) to 15.8% in Sweden [73] (Table 1.1). In Iceland, *H. pylori* prevalence was 33% (mean age, 27 years women; 1975–1997) [43]. When the seroprevalence was compared among randomly selected 25- to 50-year-old people from Estonia, Iceland, and Sweden, it was found to be 69%, 36%, and 11%, respectively [86]. In Finland, the proportion of pregnant women infected declined to nearly half between 1983 (30.1%) and 1995 (13.1%) [37] and between 1983 (30.1%) and 2001 (19.3%) [39] (Table 1.1). *H. pylori* prevalence in adults who took part in a large population-based health survey was 65.0% (1977–1980) and 59.0% (1997–1980) [38]. In addition, *H. pylori* prevalence in the aged people who were older than 100 years was 61.0% (mean age, 101 years and 1 month; 1991) [36].

In the Czech Republic, between 2001 and 2011, the prevalence decreased from 41.7% [33] to 23.5% [34] (Table 1.1). In the case of the United Kingdom, it was 13.4% (1–84 years, 1986–1996), lower than the other countries because the children were included [87]. In France, *H. pylori* prevalence in adults was 25.4% by ELISA method IgG in saliva between 1995 and 1997 [40] (Table 1.1). Similarly, the prevalence rate was 23% in Hungary (19–23 years, 1999–2000) [88] and 39.8% in Cyprus (2013) [32] (Table 1.1). In Germany, *H. pylori* prevalence in adults was 39.3% (18–89 years, 1987–1988) [41] and 40.7% (18–79 years, 1997–1999) [42] by ELISA tests to determine IgG antibody titers in serum (Table 1.1). In Italy, a dramatic decrease in the prevalence of infection occurred over the 19-year observation period due to the improvement in socioeconomic conditions in dyspeptic Sardinian patients from 1995 to 2013 [51]. The overall prevalence of *H. pylori* infection in Sardinians was 43.8% (M: 46.6% vs. F: 42.0%; $p = 0.0001$) [51] and 51% in San Marino (20–79 years, 1990–1991) [64] (Table 1.1). In contrast, a higher prevalence of *H. pylori* was reported in Portugal, where the prevalence of *H. pylori* infection was 84.2%, with 61.7% of strains also positive for CagA [63] (Table 1.1). In addition, based on the proportion of included subjects, the incidence rate of infection was 3.6/100 person-years, showing that Portuguese rates of *H. pylori* infection remain very high in Europe [63]. Similarly, Latvia also showed a higher prevalence, 79.2% in the age group of 17–99 ($n = 3564$) [19].

1.2.1.3 North America

In the United States, *H. pylori* prevalence in adults yielded small declines between 1988 and 1991 (32.7%) [80] and 1999–2000 (27.1%) [81]. In the cross-sectional study from a Veteran's Affairs Center in the United States among patients aged 40–80 years old, the overall prevalence was 28.9%, but ethnicity was the most important factor [82] (Table 1.1). That is, *H. pylori* was highest among black males aged 50–59 (53.3%), followed by Hispanic males aged 60–69 (48.1%), and lowest in non-Hispanic white males aged 40–49 (8.2%) [82]. In a Canadian study where the presence of *H. pylori* infection was evaluated in 203 aboriginal patients with dyspepsia referred for gastroscopy, *H. pylori* infection was reported by histology in 37.9% of patients [27] (Table 1.1).

1.2.1.4 Latin America

In the late 1990s/early 2000s, the prevalence estimates were generally higher among countries in Central/South America (around 20 years, ranging from 30% in Argentina [22] to 70% in Mexico [18]; around 60 years, ranging from 70% in Chile [15] to 90% in Mexico [18]) (Table 1.1). However, in 2013, a study from Mexico showed a seroprevalence rate of 52.2% among 343 pregnant women living in rural areas in Mexico [59], which is a decreased rate than the previous high seroprevalence rate in this area [18] (Table 1.1). Similarly in Ecuador, a cross-sectional seroprevalence study during 2001–2002 showed a 63.1% seroprevalence rate [16], which is rather a decreased rate than the previous reports from Latin America (Table 1.1).

1.2.1.5 Africa

New data from African countries have been summarized in the same review article [14] (Table 1.1). Studies from Morocco and Ethiopia reported a prevalence of *H. pylori* infection of

75.5% [60] and 65.7% [35], respectively. Both studies also found a significant increase with age [35, 60], probably due to the birth cohort effect. A survey from patients with dyspepsia in Nigeria reported higher values: the prevalence was 80% when tested with histology and was even higher, reaching 93.6%, when serology was applied [61]. In the case of Lebanon, the seroprevalence study performed during 2008–2009 (*n* = 308, greater than and equal to 18 years old) showed a rate of 52.0%, which is a quite decreased rate [57] (Table 1.1).

1.2.1.6 Summary

In most countries, recent surveys yielded lower prevalence estimates in the developing countries. However, *H. pylori* prevalence in adults in Africa, Central/South America, Asia, and South/East Europe was still higher than 50%, and it is related to the birth cohort effect. In the developed countries, only small variations were observed, and *H. pylori* prevalence in adults still showed a low rate, 20–40%, but the proportion of immigrants from the high-prevalence countries affected the *H. pylori* prevalence in adults, suggesting that ethnicity became a strong predictor for *H. pylori* in the developed countries [13, 14].

1.2.2 Prevalence of *H. pylori* in Children

H. pylori infection occurs mainly during childhood, especially under the age of 5 years [52, 89, 90], and *H. pylori* prevalence in adulthood depends on infection in childhood [11]. It is important to determine the status of the current *H. pylori* infection in children including prevalence, incidence, and origin of infection because such evidence can be used to expect the incidences of *H. pylori*-related diseases in the future and can also be incorporated into a prevention strategy for GC [13]. Recently, the *H. pylori* prevalence in children quickly decreased in China.

1.2.2.1 Asia

The nationwide report performed in 1998 in South Korea shows the distribution of seroprevalence according to age as Figs. 1.3 and 1.4 [67].

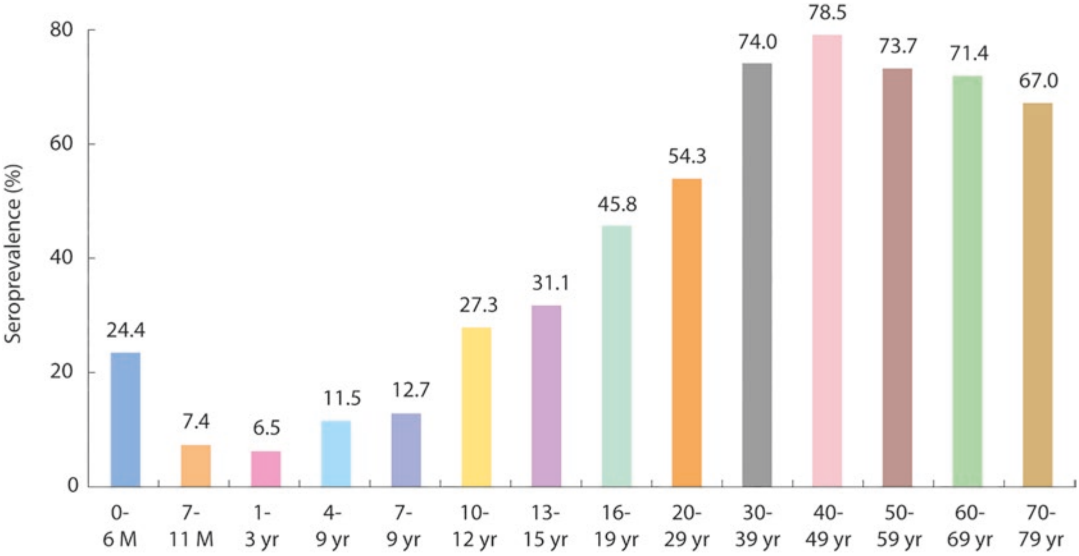

Fig. 1.3 Seroprevalence of *H. pylori* infection according to age. The seroprevalence was increased with age and was highest in people in their 40s (78.5%). The characteristic feature of our study was that the infection rate was steeply increased in three age groups (10–12 years, 16–19 years, and people in their 20s). *M* months, *yr* years (Modified from Kim et al. [67])

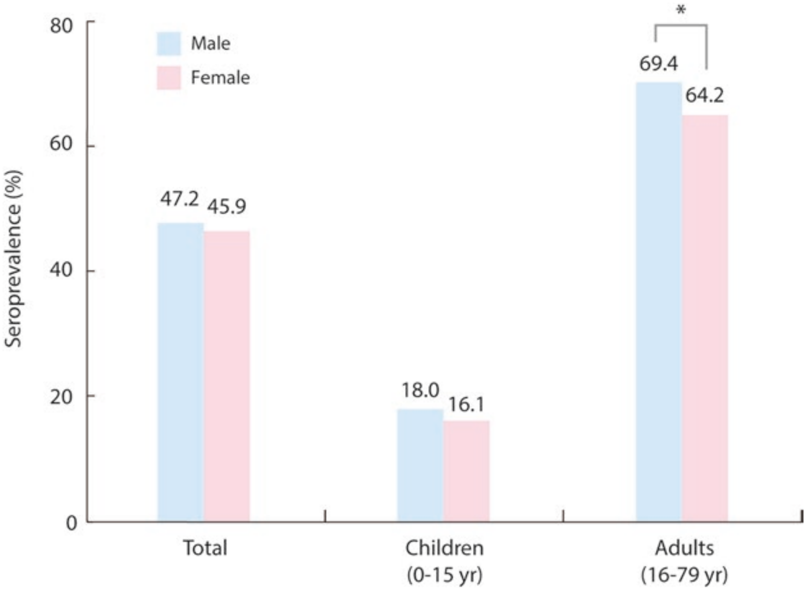

Fig. 1.4 Seroprevalence of *H. pylori* infection according to gender in children and adults. In adults, a significant difference was observed between genders. *$p < 0.05$. *yr* years (Modified from Kim et al. [67])

The seropositivity of infants from birth to 6 months was 24.4% and decreased to 6% in the group aged 1–3 years, suggesting the transfer of the mother's *H. pylori* IgG into the fetal blood [67]. The prevalence of *H. pylori* infection was increased progressively and steeply with advanced age ($p < 0.05$) from 6% in the 1- to 3-year-old group to 78.5% in the 40- to 49-year-old group. A characteristic feature of this study was that the seroprevalence increased abruptly in 10–12 years, resulting in the overall seroprevalence of *H. pylori* infection among children below 16 years old, 17.2% [67] (Table 1.2). In China, a total of 1634 children and adolescents with upper gastrointestinal (GI) symptoms, who underwent gastroscopy with gastric biopsies, were evaluated for the presence of *H. pylori* infection [93]. The histological examination of gastric biopsies showed a 32.1% prevalence of *H. pylori* infection [93]. Recently, decreasing trends have been seen in China where, in Hangzhou [94], the positivity rates were 14.8%, 20.2%, and 25.8% in 3–6, 7–11, and 12–17 years age groups, respectively, with the overall prevalence decreasing from 21.6 to 17.2% between 2007 and 2014.

In contrast, *H. pylori* prevalence in Japanese children during 2010–2011 was approximately 1.8% [99], which is much lower than that reported in Japanese adults. In detail, *H. pylori* prevalence was 1.9% among 689 children aged 0–8 years in 2010 and 1.8% among 835 children aged 0–11 in 2011 [99]. No feco-conversion was observed in 430 children aged 0–8 years (170 were aged 0–4 years) who provided follow-up stool samples after 1 year [99]. In contrast, another study in a small town in Japan showed that *H. pylori* prevalence in children was 12.1% [98] (Table 1.2), suggesting again that different environments affect the seroprevalence in one country. Recently, the trend has been similar in Japan. That is, one study from a high GC incidence area found only 85 of 1765 (4.8%) students aged 13–15 years to be positive [100], and in another, the prevalence in school children aged 12–15 years was 3.1% [101] (Table 1.2).

In the case of Iran, a higher rate of seroprevalence in children was reported, 50.5%, with 61.7% of children positive for CagA [97] (Table 1.2). In Iceland overall, 3.4% (7/205) of the children were infected with *H. pylori* [96] (Table 1.2). In Latvia on the other hand, there has been no evidence of a fall in prevalence in children over the last 10 years [102]. Furthermore, the prevalence of *H. pylori* was high, 87.7%, and it increased with population density or employee density in Vietnam [107] (Table 1.2).

Table 1.2 Prevalence of *H. pylori* infection in children

Country	Year reference	Age of included subjects (years)	Number	Diagnostic method	Prevalence of *H. pylori* (%)
Belgium	2013 [91]	12–25	516	^{13}C-UBT	11
Brazil	2014 [92]	2–19	129	Histology, RUT, culture	41.1
China	2014 [93]	1–18	1634	Histology, RUT	32.1
	2017 [94]	3–17	12,796	^{13}C-UBT	21.6– 17.2 between 2007 and 2014
Ecuador	2004 [16]	6 months–16	257	Serum IgG (not further specified)	63.0
Germany	2003 [95]	7–20	540	^{13}C-UBT	9.4
Iceland	2017 [96]	7–9	125	Serum IgG (ELISA)	3.4
Iran	2013 [97]	1–15	194	Serum IgG (ELISA)	50.5
	2016 [49]	Children		Meta-analysis (heterogenous)	42
Ireland	2005 [89]	24–48 months	327	^{13}C-UBT	8.6
Japan	2007 [90]	0–12 months	108	HpSA test	3.7
	2013 [98]	1–18	838 (389 boys and 449 girls)	Serum IgG (ELISA)	12.1
	2014 [99]	0–8/0–11	689/835	Stool antigen (PCR), UBT	1.9/1.8
	2017 [100]	13–15	1765	Urine-based ELISA	4.8
	2017 [101]	12–15	454	Serum IgG (ELISA)	3.1
Latvia	2016 [102]	1–6	101	Stool antigen test	15.5
Portugal	2013 [103]	13	1312	Serum IgG (ELISA)	66.2
South Korea	2001 [67]	0–15	2338	Serum IgG (ELISA)	17.2
Taiwan	2003 [104]	13–15	91/138	Serum IgG (ELISA)	82.4/71.0
Tunis	2003 [105]	1–15	191	Serum IgG (ELISA)	30.4
The United States	1996 [106]	6–19	2581 (1326 boys and 1255 girls)	Serum IgG (ELISA)	24.8
Vietnam	2016 [107]	6–15	1460 (730 boys and 730 girls)	Stool antigen test (EIA)	87.7

EIA enzyme immunoassay, *ELISA* enzyme-linked immunosorbent assay, *RUT* rapid urease test, *PCR* polymerase chain reaction, *HpSA H. pylori* stool antigen, *UBT* urea breath test

1.2.2.2 Europe

In Belgium, a study carried out on children and young adults reported a prevalence of infection of 11%, ranging from 3.2% in Belgian-born children to 60% in children born of foreign parents coming from countries with a high prevalence of *H. pylori* infection [91]. Similarly, the seroprevalence of German children in Germany was 13.1%, but that of Turkish children in Germany and Turkish children in Turkey was 30.4% and 44.5%, respectively [77], suggesting that seroprevalence could be different depending on race and socioeconomic status. Similarly, 7- to 9-year-old German children showed 9.4% of *H. pylori* prevalence by UBT [95], but a subgroup analysis showed that German children prevalence was 7.1% and that of the immigrant children was 28.2% [95] (Table 1.2). In contrast, Bastos et al. reported a very high prevalence of infection in Portuguese children [103], similar to the high prevalence in adults [63]. Among 13-year-old students from Porto, the prevalence was 66.2% (Table 1.2). More than half of the negative subjects were again tested after a median follow-up of 37 months, revealing an incidence rate of 4.1/100 person-years [103].

1.2.2.3 North America

In the United States, *H. pylori* prevalence in children and adolescents was 24.8% (6–19 years, 1988–1991) [106] (Table 1.2).

1.2.2.4 Latin America

In Brazil, Pacheco et al. compared several diagnostic tests and reported a high prevalence of 41.1% in subjects aged 2.1–19 years old [92] (Table 1.2).

1.2.2.5 Summary

H. pylori prevalence in children is very variable in the world, suggesting that still active *H. pylori* infection occurs in childhood in some countries.

1.3 Risk Factors of *H. pylori* Infection

There have been many studies regarding the risk factors of *H. pylori*. The most frequent independent risk factors for *H. pylori* infection were living in rural areas, poor sanitation, overcrowding, lower educational level, and low socioeconomic status [67]. In terms of gender, most studies reported no significant difference in *H. pylori* infection between men and women, both in adults [25, 26, 35, 45, 60, 74] and in children [67, 91, 103]. However, in South Korea, gender showed no difference in *H. pylori* prevalence among children, but the male gender became a risk factor in adults aged 13 years and over [8, 20, 67] (Table 1.3). In contrast, women showed higher *H. pylori* prevalence in Iran [48], suggesting that somehow intimate personal relationships could be related to *H. pylori* transmission. In terms of age, *H. pylori* seroprevalence became definitely higher in the adults than in the children recently [99]. However, no significant association was found between infection and age in the adult population [26, 44, 45, 75]. Instead, the age-specific gradient in *H. pylori* prevalence reported by some studies seems to be related to a birth cohort effect [8, 35, 60, 74, 93]. Several socioeconomic factors have been associated with *H. pylori* infection [14]. In particular, subjects with a low socioeconomic status, which has been measured as a low family income, had a higher likelihood of carrying *H. pylori* infection [30, 75], especially in the childhood [65] (Table 1.3). Furthermore, an inverse association between educational level and *H. pylori* infection was found in the majority

Table 1.3 Multivariate analysis of risk factors for *H. pylori* infection in three nationwide epidemiologic studies in South Korea

	Children (1–15 years)	Adults (≥16 years)
2001 [65]	Age	Age
	Geographic areas	Geographic areas
	Drinking water (tap or well water)	Sex (male)
	Low mother's education	Crowding in childhood
	Family income per month	Low economic status in childhood
2007 [20]		Age
		Geographic areas
		Sex (male)
		Medium or low monthly income
		Medium education than high
2013 [8]		Age
		Geographic area
		Sex (male)
		Medium or low monthly income
		Medium education than high cholesterol (240 ≥ mg/dL)
2016–2017 [9]		Age
		Geographic area
		Sex (male)
		Medium monthly income vs. high (more than US $ 10,000 per month)
		Medium education level vs. high (university graduates or graduates of postgraduate course

of the studies [8, 20, 60, 63, 78]; except for two cases [30, 48], individuals with lower educational levels had a higher risk than those with higher education [14]. The same association concerning the parents' education was also found in studies on children [91, 103], especially the mother's education [65] (Table 1.3). Moreover, several factors related to residence have been found to be associated with the infection [14]. Indeed, living in a rural area [20, 25] and in crowded homes [26, 65, 103], and having contaminated sources of drinking water [65, 78] were risk factors for *H. pylori* infection (Table 1.3). Among the main lifestyle habits, smoking and alcohol consumption showed discordant results [14]. Although in most studies, there was no significant association with *H. pylori* infection [8, 20, 30, 60, 65, 75], some authors reported that regular smokers in Turkey [78] and in Saudi Arabia [108] and drinkers in Saudi Arabia [108] were at higher risk [14]. In contrast, in one study, regular alcohol drinking was a protective factor for *H. pylori* infection in Turkey [78]. The main subpopulations in Malaysia are Malays, Chinese, and Indians who have a *H. pylori* prevalence of 16.4%, 48.1%, and 61.8%, respectively. However, three Aboriginal tribes (the Orang Asli) comprise 1% of the Malaysian population and are divided into three separate tribes (the Negrito, Senoi, and Proto-Malay) and live in seven isolated settlements. A total of 275 subjects were studied, 115 (44.7%) were *H. pylori* positive, the highest seroprevalence being among Negrito (65.7%), and prevalence appeared to be related to age, gender, tribe, and house materials [58].

1.4 Transmission of *H. pylori*

It has now been established that *H. pylori* is mainly transmitted through person-to-person, especially in the childhood and intrafamilial transmission [55, 109–112]. However, it is not clear how the bacteria are transmitted from person to person and why the colonization does not occur in some persons, but it persists forever in others. The main route of transmission is regarded through oral–oral, fecal–oral, or gastric–oral. As the route of transmission could be different depending on developing or developed countries because the exposed age, race, and socioeconomic status are variable in these countries [105], the transmission of *H. pylori* was summarized in two conditions of the developing and developed countries in this chapter.

1.4.1 Transmission of *H. pylori* in the Developing Countries

In the past, fecal–oral transmission was regarded as the main route in the developing countries. That is, environmental transmission, such as drinking contaminated water, could play a major role. However, as more evidence came out, fecal–oral transmission became a minor route although it remains a possible route. In addition, the transmission of *H. pylori* was found to be different from hepatitis A viral infection [76, 104, 113]. Instead, parental transmission has been frequently reported. Didelot et al. sequenced the genomes of 97 *H. pylori* isolates from 52 members of two families living in rural conditions in South Africa [79]. Transmission events were more frequent between close relatives and between individuals living in the same house. Turkish report has shown a close relationship of colonized *H. pylori* between the dental plaque and gastric epithelium [77]. In addition, further evidence came from Brazil that an *H. pylori*-infected mother was a strong and independent risk factor (odds ratio 22.7; 95% confidence interval, 2.31–223.21) [114]. Taken together, oral–oral transmission became a more important transmission route of *H. pylori* infection even in developing countries. This could be related to the characteristics of *H. pylori*, which need good conditions such as humidity and anaerobic conditions with warmth.

1.4.2 Transmission of *H. pylori* in the Developed Countries

In developed countries, parental transmission has been frequently reported [99], but the transmission route is not so simple because of the immigrants from countries with a high prevalence of *H. pylori*. For instance, the seroprevalence of German children in Germany was 13.1%, but that of Turkish children in Germany was 30.4%, and Turkish in Turkey was 44.5% [77], suggesting that seroprevalence could be different depending on race and socioeconomic status. Similarly, among 7- to 9-year-old Germans, *H. pylori* prevalence was 9.4% by UBT, but a subgroup analysis showed that prevalence in German children was 7.1% and that of the immigrants' children was 28.2% [95]. In this study, the number of family members was found to be a risk factor, and the prevalence rate was high when a family member had GI symptoms [95]. The intrafamilial transmission became strong from much evidence in the developed countries. One study that performed fingerprinting showed that among 35 families, the strain of 29 families (81%) was the same [111]. In addition, the concordance between mother and children was 56% (10 among 18 families), but none between father and children, and 22% between husband and wife, suggesting that a close relationship increases the possibility of transmission [111]. Osaki et al. performed a multilocus sequence typing DNA analysis using the stools of parents belonging to three families with a child positive for *H. pylori* infection [115]. The study showed an intrafamilial transmission in all selected families, with a mother-to-child transmission in at least two families. Similarly, Urita et al. investigated the intrafamilial transmission of *H. pylori* infection by testing 838 children and their family members from a small town in Japan [98]. The *H. pylori* prevalence in children was 12.1%, and most risk factors were the siblings, mother, and grandmother, but the father and grandfather were not a risk factor [98]. Indeed, it seems that mothers transmit the infection through mouth secretions, using common spoons, or tasting the child's food [14]. Grandmothers might take care of their grandchildren when mothers are at work increasing the risk of transmission. A similar report has come out from Ireland that children could be infected when the mother or siblings had *H. pylori* infection [89]. Taken together, *H. pylori* infection in the developed countries could have characteristics of developing countries due to the immigrants from countries with a high prevalence of *H. pylori*. However, the oral–oral especially mother-to-child transmission is the main route.

1.5 Conclusion

Data from recent studies show that the prevalence of *H. pylori* infection is still high in most countries worldwide. However, the decrease is very prominent when the countries are in the transition from developing to developed countries such as South Korea and urban areas of China. *H. pylori* seems to be less frequent in Northern European and North American populations; however, about one-third of the adults seem to still be infected. Even in these countries, *H. pylori* remains highly prevalent in immigrants coming from countries with a high prevalence of *H. pylori*. The most frequent independent risk factors for *H. pylori* infection were living in rural areas, poor sanitation, overcrowding, lower educational level, and low socioeconomic status. Parent-to-child infection is thought to be the main infection route of infrequent infection for children. The lower prevalence of infection in the younger generations suggests a further decline in *H. pylori* prevalence in the community over the coming decades.

References

1. Egan BJ, Holmes K, O'Connor HJ, O'Morain CA. *Helicobacter pylori* gastritis, the unifying concept for gastric diseases. Helicobacter. 2007;12 (Suppl 2):39–44.
2. Parsonnet J. The incidence of *Helicobacter pylori* infection. Aliment Pharmacol Ther. 1995;9 (Suppl 2):45–51.
3. Roosendaal R, Kuipers EJ, Buitenwerf J, van Uffelen C, Meuwissen SG, van Kamp GJ, et al. *Helicobacter pylori* and the birth cohort effect: evidence of a continuous decrease of infection rates in childhood. Am J Gastroenterol. 1997;92:1480–2.
4. Burucoa C, Axon A. Epidemiology of *Helicobacter pylori* infection. Helicobacter. 2017;22 (Suppl 1):e12403.

5. Fuccio L, Eusebi LH, Bazzoli F. Gastric cancer, *Helicobacter pylori* infection and other risk factors. World J Gastrointest Oncol. 2010;2:342–7.
6. Sonnenberg A. Review article: historic changes of *Helicobacter pylori*-associated diseases. Aliment Pharmacol Ther. 2013;38:329–42.
7. Lim JH, Kim N, Lim SH, Kwon JW, Shin CM, Chang YS, et al. Inverse relationship between *Helicobacter pylori* infection and asthma among adults younger than 40 years: a cross-sectional study. Medicine (Baltimore). 2016;95:e2609.
8. Lim SH, Kwon JW, Kim N, Kim GH, Kang JM, Park MJ, et al. Prevalence and risk factors of *Helicobacter pylori* infection in Korea: nationwide multicenter study over 13 years. BMC Gastroenterol. 2013;13:104.
9. Lim SH, Kim N, Kwon JW, Kim SE, Baik GH, Lee JY, et al. Trends in the seroprevalence of *Helicobacter pylori* infection and its putative eradication rate over 18 years in Korea: a cross-sectional nationwide multicenter study. PLoS One. 2018;13:e0204762.
10. Veldhuyzen van Zanten SJ, Pollak PT, Best LM, Bezanson GS, Marrie T. Increasing prevalence of *Helicobacter pylori* infection with age: continuous risk of infection in adults rather than cohort effect. J Infect Dis. 1994;169:434–7.
11. Banatvala N, Mayo K, Megraud F, Jennings R, Deeks JJ, Feldman RA. The cohort effect and *Helicobacter pylori*. J Infect Dis. 1993;168:219–21.
12. Kosunen TU, Aromaa A, Knekt P, Salomaa A, Rautelin H, Lohi P, et al. *Helicobacter* antibodies in 1973 and 1994 in the adult population of Vammala, Finland. Epidemiol Infect. 1997;119:29–34.
13. Peleteiro B, Bastos A, Ferro A, Lunet N. Prevalence of *Helicobacter pylori* infection worldwide: a systematic review of studies with national coverage. Dig Dis Sci. 2014;59:1698–709.
14. Eusebi LH, Zagari RM, Bazzoli F. Epidemiology of *Helicobacter pylori* infection. Helicobacter. 2014;19 (Suppl 1):1–5.
15. Ferreccio C, Rollán A, Harris PR, Serrano C, Gederlini A, Margozzini P, et al. Gastric cancer is related to early *Helicobacter pylori* infection in a high-prevalence country. Cancer Epidemiol Biomarkers Prev. 2007;16:662–7.
16. Gómez NA, Salvador A, Vargas PE, Zapatier JA, Alvarez J. Seroprevalence of *Helicobacter pylori* among the child population of Ecuador. Rev Gastroenterol Peru. 2004;24:230–3.
17. Shiota S, Murakami K, Fujioka T, Yamaoka Y. Population-based strategies for *Helicobacter pylori*-associated disease management: a Japanese perspective. Expert Rev Gastroenterol Hepatol. 2010;4:149–56.
18. Torres J, Leal-Herrera Y, Perez-Perez G, Gomez A, Camorlinga-Ponce M, Cedillo-Rivera R, et al. A community-based seroepidemiologic study of *Helicobacter pylori* infection in Mexico. J Infect Dis. 1998;178:1089–94.
19. Leja M, Cine E, Rudzite D, Vilkoite I, Huttunen T, Daugule I, et al. Prevalence of *Helicobacter pylori* infection and atrophic gastritis in Latvia. Eur J Gastroenterol Hepatol. 2012;24:1410–7.
20. Yim JY, Kim N, Choi SH, Kim YS, Cho KR, Kim SS, et al. Seroprevalence of *Helicobacter pylori* in South Korea. Helicobacter. 2007;12:333–40.
21. Wang X, Terry P, Yan H. Stomach cancer in 67 Chinese counties: evidence of interaction between salt consumption and *Helicobacter pylori* infection. Asia Pac J Clin Nutr. 2008;17:644–50.
22. Olmos JA, Rios H, Higa R. Prevalence of *Helicobacter pylori* infection in Argentina: results of a nationwide epidemiologic study. Argentinean HP Epidemiologic Study Group. J Clin Gastroenterol. 2000;31:33–7.
23. Moujaber T, MacIntyre CR, Backhouse J, Gidding H, Quinn H, Gilbert GL. The seroepidemiology of *Helicobacter pylori* infection in Australia. Int J Infect Dis. 2008;12:500–4.
24. Pandeya N, Whiteman DC, Australian Cancer Study. Prevalence and determinants of *Helicobacter pylori* sero-positivity in the Australian adult community. J Gastroenterol Hepatol. 2011;26:1283–9.
25. Vilaichone RK, Mahachai V, Shiota S, Uchida T, Ratanachu-ek T, Tshering L, et al. Extremely high prevalence of *Helicobacter pylori* infection in Bhutan. World J Gastroenterol. 2013;19:2806–10.
26. Dorji D, Dendup T, Malaty HM, Wangchuk K, Yangzom D, Richter JM. Epidemiology of *Helicobacter pylori* in Bhutan: the role of environment and geographic location. Helicobacter. 2014;19:69–73.
27. Sethi A, Chaudhuri M, Kelly L, Hopman W. Prevalence of *Helicobacter pylori* in a first nations population in northwestern Ontario. Can Fam Physician. 2013;59:e182–7.
28. Shi R, Xu S, Zhang H, Ding Y, Sun G, Huang X, et al. Prevalence and risk factors for *Helicobacter pylori* infection in Chinese populations. Helicobacter. 2008;13:157–65.
29. Cheng H, Hu FL, Zhang L, Yang G, Ma J, Hu J, et al. Prevalence of *Helicobacter pylori* infection and identification of risk factors in rural and urban Beijing, China. Helicobacter. 2009;14:128–33.
30. Zhu Y, Zhou X, Wu J, Su J, Zhang G. Risk factors and prevalence of *Helicobacter pylori* infection in persistent high incidence area of gastric carcinoma in Yangzhong city. Gastroenterol Res Pract. 2014;2014:481365.
31. Yu X, Yang X, Yang T, Dong Q, Wang L, Feng L. Decreasing prevalence of *Helicobacter pylori* according to birth cohorts in urban China. Turk J Gastroenterol. 2017;28:94–7.
32. Krashias G, Bashiardes S, Potamitou A, Potamitis GS, Christodoulou C. Prevalence of *Helicobacter pylori* cagA and vacA genes in Cypriot patients. J Infect Dev Ctries. 2013;7:642–50.
33. Bures J, Kopácová M, Koupil I, Vorísek V, Rejchrt S, Beránek M, et al. Epidemiology of *Helicobacter*

pylori infection in the Czech Republic. Helicobacter. 2006;11:56–65.

34. Bures J, Kopáčová M, Koupil I, Seifert B, Skodová Fendrichová M, et al. Significant decrease in prevalence of *Helicobacter pylori* in the Czech Republic. World J Gastroenterol. 2012;18:4412–8.
35. Mathewos B, Moges B, Dagnew M. Seroprevalence and trend of *Helicobacter pylori* infection in Gondar University Hospital among dyspeptic patients, Gondar, North West Ethiopia. BMC Res Notes. 2013;6:346.
36. Rehnberg-Laiho L, Louhija J, Rautelin H, Jusufovic J, Tilvis R, Miettinen A, et al. *Helicobacter* antibodies in Finnish centenarians. J Gerontol A Biol Sci Med Sci. 1999;54:M400–3.
37. Rehnberg-Laiho L, Rautelin H, Koskela P, Sarna S, Pukkala E, Aromaa A, et al. Decreasing prevalence of *Helicobacter* antibodies in Finland, with reference to the decreasing incidence of gastric cancer. Epidemiol Infect. 2001;126:37–42.
38. Salomaa-Räsänen A, Kosunen TU, Karjalainen J, Aromaa A, Knekt P, Sarna S, et al. IgA antibodies in persisting *Helicobacter pylori* infection in Finnish adults. Clin Microbiol Infect. 2006;12:236–40.
39. Seiskari T, Viskari H, Kaila M, Haapala AM, Koskela P, Hyöty H. Time trends in allergic sensitisation and *Helicobacter pylori* prevalence in Finnish pregnant women. Int Arch Allergy Immunol. 2009;150:83–8.
40. Broutet N, Sarasqueta AM, Cantet F, Lethuaire D, Mégraud F. Is there a link between the variation in gastric cancer mortality and differences in *Helicobacter pylori* prevalence in different regions of France? Gastroenterol Clin Biol. 1999;23:754–60.
41. Brenner H, Berg G, Frohlich M, Boeing H, Koenig W. Chronic infection with *Helicobacter pylori* does not provoke major systemic inflammation in healthy adults: results from a large population-based study. Atherosclerosis. 1999;147:399–403.
42. Kuepper-Nybelen J, Thefeld W, Rothenbacher D, Brenner H. Patterns of alcohol consumption and *Helicobacter pylori* infection: results of a population-based study from Germany among 6545 adults. Aliment Pharmacol Ther. 2005;21:57–64.
43. Lehtinen M, Ogmundsdottir HM, Bloigu A, Hakulinen T, Hemminki E, Gudnadottir M, et al. Associations between three types of maternal bacterial infection and risk of leukemia in the offspring. Am J Epidemiol. 2005;162:662–7.
44. Sodhi JS, Javid G, Zargar SA, Tufail S, Shah A, Khan BA, et al. Prevalence of *Helicobacter pylori* infection and the effect of its eradication on symptoms of functional dyspepsia in Kashmir, India. J Gastroenterol Hepatol. 2013;28:808–13.
45. Adlekha S, Chadha T, Krishnan P, Sumangala B. Prevalence of *Helicobacter pylori* infection among patients undergoing upper gastrointestinal endoscopy in a medical college hospital in Kerala, India. Ann Med Health Sci Res. 2013;3:559–63.
46. Syam AF, Miftahussurur M, Makmun D, Nusi IA, Zain LH, Zulkhairi, Akil F, et al. Risk factors and prevalence of *Helicobacter pylori* in five largest islands of Indonesia: a preliminary study. PLoS One. 2015;10:e0140186.
47. Goto Y, Syam AF, Darnindro N, Hapsari FPC. Risk factors for and prevalence of *Helicobacter pylori* infection among healthy inhabitants in northern Jakarta, Indonesia. Asian Pac J Cancer Prev. 2016;17:4469–75.
48. Eshraghian A. Epidemiology of *Helicobacter pylori* infection among the healthy population in Iran and countries of the Eastern Mediterranean Region: a systematic review of prevalence and risk factors. World J Gastroenterol. 2014;20:17618–25.
49. Moosazadeh M, Lankarani KB, Afshari M. Meta-analysis of the prevalence of *Helicobacter pylori* infection among children and adults of Iran. Int J Prev Med. 2016;7:48.
50. Gdalevich M, Cohen D, Ashkenazi I, Mimouni D, Shpilberg O, Kark JD. *Helicobacter pylori* infection and subsequent peptic duodenal disease among young adults. Int J Epidemiol. 2000;29:592–5.
51. Dore MP, Marras G, Rocchi C, Soro S, Loria MF, Bassotti G, et al. Changing prevalence of *Helicobacter pylori* infection and peptic ulcer among dyspeptic Sardinian patients. Intern Emerg Med. 2015;10:787–94.
52. Konno M, Fujii N, Yokota S, Sato K, Takahashi M, Sato K, et al. Five-year follow-up study of mother-to-child transmission of *Helicobacter pylori* infection detected by a random amplified polymorphic DNA fingerprinting method. J Clin Microbiol. 2005;43:2246–50.
53. Kikuchi S, Yagyu K, Obata Y, Yingsong L, Yatsuya H, Hoshiyama Y, et al. Serum pepsinogen values and *Helicobacter pylori* status among control subjects of a nested case-control study in the JACC study. J Epidemiol. 2005;15 (Suppl 2):S126–33.
54. Sasazuki S, Inoue M, Iwasaki M, Otani T, Yamamoto S, Ikeda S, et al. Effect of *Helicobacter pylori* infection combined with *cagA* and pepsinogen status on gastric cancer development among Japanese men and women: a nested case-control study. Cancer Epidemiol Biomarkers Prev. 2006;15:1341–7.
55. Raymond J, Thiberg JM, Chevalier C, Kalach N, Bergeret M, Labigne A, et al. Genetic and transmission analysis of *Helicobacter pylori* strains within a family. Emerg Infect Dis. 2004;10:1816–21.
56. Benberin V, Bektayeva R, Karabayeva R, Lebedev A, Akemeyeva K, Paloheimo L, et al. Prevalence of *H. pylori* infection and atrophic gastritis among symptomatic and dyspeptic adults in Kazakhstan. A hospital-based screening study using a panel of serum biomarkers. Anticancer Res. 2013;33:4595–602.
57. Naja F, Nasreddine L, Hwalla N, Moghames P, Shoaib H, Fatfat M, et al. Association of *H. pylori* infection with insulin resistance and metabolic

syndrome among Lebanese adults. Helicobacter. 2012;17:444–51.

58. Thevakumar K, Chandren JR, Perez-Perez GI, Chua EG, Teh LK, Salleh MZ, et al. Assessment of risk and sero-prevalence of *Helicobacter pylori* colonization among remote Orang Asli tribes in peninsula Malaysia. PLoS One. 2016;11:e0159830.
59. Alvarado-Esquivel C. Seroepidemiology of *Helicobacter pylori* infection in pregnant women in rural Durango. Int J Biomed Sci. 2013;9:224–9.
60. Benajah DA, Lahbabi M, Alaoui S, El Rhazi K, El Abkari M, Nejjari C, et al. Prevalence of *Helicobacter pylori* and its recurrence after successful eradication in a developing nation (Morocco). Clin Res Hepatol Gastroenterol. 2013;37:519–26.
61. Olokoba AB, Gashau W, Bwala S, Adamu A, Salawu FK. *Helicobacter pylori* infection in Nigerians with dyspepsia. Ghana Med J. 2013;47:79–81.
62. Al-Balushi MS, Al-Busaidi JZ, Al-Daihani MS, Shafeeq MO, Hasson SS. Sero-prevalence of *Helicobacter pylori* infection among asymptomatic healthy Omani blood donors. Asia Pac J Trop Dis. 2013;3:146–9.
63. Bastos J, Peleteiro B, Barros R, Alves L, Severo M, de Fátima PM, et al. Sociodemographic determinants of prevalence and incidence of *Helicobacter pylori* infection in Portuguese adults. Helicobacter. 2013;18:413–22.
64. Gasbarrini G, Pretolani S, Bonvicini F, Gatto MR, Tonelli E, Mégraud F, et al. A population based study of *Helicobacter pylori* infection in a European country: the San Marino study. Relations with gastrointestinal diseases. Gut. 1995;36:838–44.
65. Kim N, Kim JG, Kim JH, Kim HY, Kim SW, Kim JJ, et al. Risk factors of *Helicobacter pylori* infection in asymptomatic Korean population. Korean J Med. 2000;59:376–87.
66. Chua TS, Fock KM, Chan YH, Dhamodaran S, Sim CS, Ng TM, et al. Seroreactivity to 19.5-kDa antigen in combination with absence of seroreactivity to 35-kDa antigen is associated with an increased risk of gastric adenocarcinoma. Helicobacter. 2002;7:257–64.
67. Kim JH, Kim HY, Kim NY, Kim SW, Kim JG, Kim JJ, et al. Seroepidemiological study of *Helicobacter pylori* infection in asymptomatic people in South Korea. J Gastroenterol Hepatol. 2001;16:969–75.
68. Shin A, Shin HR, Kang D, Park SK, Kim CS, Yoo KY. A nested case-control study of the association of *Helicobacter pylori* infection with gastric adenocarcinoma in Korea. Br J Cancer. 2005;92:1273–5.
69. Kim N, Lee SW, Cho SI, Park CG, Yang CH, Kim HS, et al. The prevalence of and risk factors for erosive oesophagitis and non-erosive reflux disease: a nationwide multicentre prospective study in Korea. Aliment Pharmacol Ther. 2008;27:173–85.
70. Sörberg M, Nyrén O, Granström M. Unexpected decrease with age of *Helicobacter pylori* seroprevalence among Swedish blood donors. J Clin Microbiol. 2003;41:4038–42.
71. Ye W, Held M, Lagergren J, Engstrand L, Blot WJ, McLaughlin JK, et al. *Helicobacter pylori* infection and gastric atrophy: risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. J Natl Cancer Inst. 2004;96:388–96.
72. Persson C, Jia Y, Pettersson H, Dillner J, Nyrén O, Ye W. *H. pylori* seropositivity before age 40 and subsequent risk of stomach cancer: a glimpse of the true relationship? PLoS One. 2011;6:e17404.
73. Agréus L, Hellström PL, Talley NJ, Wallner B, Forsberg A, Vieth M, et al. A towards a healthy stomach? *Helicobacter pylori* prevalence has dramatically decreased over 23 years in adults in a Swedish community. United European Gastroenterol J. 2016;4:686–96.
74. van Blankenstein M, van Vuuren AJ, Looman CW, Ouwendijk M, Kuipers EJ. The prevalence of *Helicobacter pylori* infection in the Netherlands. Scand J Gastroenterol. 2013;48:794–800.
75. den Hollander WJ, Holster IL, den Hoed CM, van Deurzen F, van Vuuren AJ, Jaddoe VW, et al. Ethnicity is a strong predictor for *Helicobacter pylori* infection in young women in a multiethnic European city. J Gastroenterol Hepatol. 2013;28:1705–11.
76. Maherzi A, Bouaziz Abed A, Fendri C, Oubich F, Koubaa C, Fauchere JL, et al. *Helicobacter pylori* infection: prospective study for asymptomatic Tunisian children. Arch Pediatr. 2003;10:204–7.
77. Porsch-Ozcürümez M, Doppl W, Hardt PD, Schnell-Kretschmer H, Tuncay M, Akinci A, et al. Impact of migration on *Helicobacter pylori* seroprevalence in the offspring of Turkish immigrants in Germany. Turk J Pediatr. 2003;45:203–8.
78. Ozaydin N, Turkyilmaz SA, Cali S. Prevalence and risk factors of *Helicobacter pylori* in Turkey: a nationally-representative, cross-sectional, screening with the ^{13}C-Urea breath test. BMC Public Health. 2013;13:1215.
79. Didelot X, Nell S, Yang I, Woltemate S, van der Merwe S, Suerbaum S. Genomic evolution and transmission of *Helicobacter pylori* in two South African families. Proc Natl Acad Sci U S A. 2013;110:13880–5.
80. Everhart JE, Kruszon-Moran D, Perez-Perez GI, Tralka TS, McQuillan G. Seroprevalence and ethnic differences in *Helicobacter pylori* infection among adults in the United States. J Infect Dis. 2000;181:1359–63.
81. Cardenas VM, Mulla ZD, Ortiz M, Graham DY. Iron deficiency and *Helicobacter pylori* infection in the United States. Am J Epidemiol. 2006;163:127–34.
82. Nguyen T, Ramsey D, Graham D, Shaib Y, Shiota S, Velez M, et al. The prevalence of *Helicobacter pylori*

remains high in African American and Hispanic veterans. Helicobacter. 2015;20:305–15.

83. Asaka M, Kimura T, Kudo M, Takeda H, Mitani S, Miyazaki T, et al. Relationship of *Helicobacter pylori* to serum pepsinogens in an asymptomatic Japanese population. Gastroenterology. 1992;102:760–6.
84. Shiota S, Murakawi K, Suzuki R, Fujioka T, Yamaoka Y. *Helicobacter pylori* infection in Japan. Expert Rev Gastroenterol Hepatol. 2013;7:35–40.
85. McDonald AM, Sarfati D, Baker MG, Blakely T. Trends in *Helicobacter pylori* infection among Maori, Pacific, and European birth cohorts in New Zealand. Helicobacter. 2014;20:139–45.
86. Thjodleifsson B, Asbjörnsdottir H, Sigurjonsdottir RB, Gíslason D, Olafsson I, Cook E, et al. Seroprevalence of *Helicobacter pylori* and cagA antibodies in Iceland, Estonia and Sweden. Scand J Infect Dis. 2007;39:683–9.
87. Vyse AJ, Gay NJ, Hesketh LM, Andrews NJ, Marshall B, Thomas HI, et al. The burden of *Helicobacter pylori* infection in England and Wales. Epidemiol Infect. 2002;128:411–7.
88. Fürész J, Lakatos S, Németh K, Fritz P, Simon L, Kacserka K. The prevalence and incidence of *Helicobacter pylori* infections among young recruits during service in the Hungarian army. Helicobacter. 2004;9:77–80.
89. Rowland M, Daly L, Vaughan M, Higgins A, Bourke B, Drumm B. Age-specific incidence of *Helicobacter pylori*. Gastroenterology. 2006;130:65–72.
90. Okuda M, Miyashiro E, Booka M, Tsuji T, Nakazawa T. *Helicobacter pylori* colonization in the first 3 years of life in Japanese children. Helicobacter. 2007;12:324–7.
91. Mana F, Vandebosch S, Miendje Deyi V, Haentjens P, Urbain D. Prevalence of and risk factors for *H. pylori* infection in healthy children and young adults in Belgium anno 2010/2011. Acta Gastroenterol Belg. 2013;76:381–5.
92. Pacheco SL, Ogata SK, Machado RS, Patricio FR, Pardo ML, Kawakami E. Diagnosis of *Helicobacter pylori* infection by means of reduced-dose (13)C-urea breath test and early sampling of exhaled breath. J Pediatr Gastroenterol Nutr. 2013;57:607–11.
93. Yu Y, Su L, Wang X, Wang X, Xu C. Association between *Helicobacter pylori* infection and pathological changes in the gastric mucosa in Chinese children. Intern Med. 2014;53:83–8.
94. Shu X, Ping M, Yin G, Jiang M. Investigation of *Helicobacter pylori* infection among symptomatic children in Hangzhou from 2007 to 2014: a retrospective study with 12,796 cases. PeerJ. 2017;5:e2937.
95. Grimm W, Fischbach W. *Helicobacter pylori* infection in children and juveniles: an epidemiological study on prevalence, socio-economic factors and symptoms. Dtsch Med Wochenschr. 2003;128:1878–83.
96. Asgeirsdottir GA, Kjartansdottir I, Olafsdottir AS, Hreinsson JP, Hrafnkelsson H, Johannsson E, et al. *Helicobacter pylori* infection in Icelandic children. Scand J Gastroenterol. 2017;52:686–90.
97. Ghasemi-Kebria F, Ghaemi E, Azadfar S, Roshandel G. Epidemiology of *Helicobacter pylori* infection among Iranian children. Arab J Gastroenterol. 2013;14:169–72.
98. Urita Y, Watanabe T, Kawagoe N, Takemoto I, Tanaka H, Kijima S, et al. Role of infected grandmothers in transmission of *Helicobacter pylori* to children in a Japanese rural town. J Paediatr Child Health. 2013;49:394–8.
99. Okuda M, Osaki T, Lin Y, Yonezawa H, Maekawa K, Kamiya S, et al. Low prevalence and incidence of *Helicobacter pylori* infection in children: a population-based study in Japan. Helicobacter. 2015;20:133–8.
100. Kusano C, Gotoda T, Ishikawa H, Moriyama M. The administrative project of *Helicobacter pylori* infection screening among junior high school students in an area of Japan with a high incidence of gastric cancer. Gastric Cancer. 2017;20:16–9.
101. Nakayama Y, Lin Y, Hongo M, Hidaka H, Kikuchi S. *Helicobacter pylori* infection and its related factors in junior high school students in Nagano Prefecture, Japan. Helicobacter. 2017;22:e12363.
102. Daugule I, Karklina D, Rudzite D, Remberga S, Rumba-Rozenfelde I. Prevalence of *Helicobacter pylori* infection among preschool children in Latvia: no significant decrease in prevalence during a ten year period. Scand J Public Health. 2016;44:418–22.
103. Bastos J, Peleteiro B, Pinto H, Marinho A, Guimarães JT, Ramos E, et al. Prevalence, incidence and risk factors for *Helicobacter pylori* infection in a cohort of Portuguese adolescents (EpiTeen). Dig Liver Dis. 2013;45:290–5.
104. Yang YJ, Wang SM, Chen CT, Huang MC, Chang CJ, Liu CC. Lack of evidence for fecal-oral transmission of *Helicobacter pylori* infection in Taiwanese. J Formos Med Assoc. 2003;102:375–8.
105. Suerbaum S, Michetti P. *Helicobacter pylori* infection. N Engl J Med. 2002;347:1175–86.
106. Staat MA, Kruszon-Moran D, McQuillan GM, Kaslow RA. A population-based serologic survey of *Helicobacter pylori* infection in children and adolescents in the United States. J Infect Dis. 1996;174:1120–3.
107. Nguyen TVH, Nguyen VB, Phan TTB, Nguyen HT, Vo KT, Ngo XM, et al. Epidemiology of *Helicobacter pylori* infection in Tay children in Vietnam. Ann Clin Lab Res. 2016;4:125.
108. Hanafi MI, Mohamed AM. *Helicobacter pylori* infection: seroprevalence and predictors among healthy individuals in Al Madinah, Saudi Arabia. J Egypt Public Health Assoc. 2013;88:40–5.
109. Drumm B, Perez-Perez GI, Blaser MJ, Sherman PM. Intrafamilial clustering of *Helicobacter pylori* infection. N Engl J Med. 1990;322:359–63.
110. Goodman KJ, Correa P. Transmission of *Helicobacter pylori* among siblings. Lancet. 2000;355:358–62.

111. Kivi M, Tindberg Y, Sōrberg M, Casswall TH, Befrits R, Hellström PM, et al. Concordance of *Helicobacter pylori* strains within families. J Clin Microbiol. 2003;41:5604–8.
112. Fujimoto Y, Furusyo N, Toyoda K, Takeoka H, Sawayama Y, Hayashi J. Intrafamilial transmission of *Helicobacter pylori* among the population of endemic areas in Japan. Helicobacter. 2007;12:170–6.
113. Chen LK, Hwang SJ, Wu TC, Chu CH, Shaw CK. *Helicobacter pylori* and hepatitis A virus infection in school-aged children on two isolated neighborhood islands in Taiwan. Helicobacter. 2003;8:168–72.
114. Pérez-Pérez GI, Sack RB, Reid R, Santosham M, Croll J, Blaser MJ. Transient and persistent *Helicobacter pylori* colonization in native American children. J Clin Microbiol. 2003;41:2401–7.
115. Osaki T, Okuda M, Ueda J, Konno M, Yonezawa H, Hojo F, et al. Multilocus sequence typing of DNA from faecal specimens for the analysis of intra-familial transmission of *Helicobacter pylori*. J Med Microbiol. 2013;62(Pt 5):761–5.

Part II

Pathophysiology

2 Gastric Colonization by *H. pylori*

Elizabeth A. Marcus and David R. Scott

Abstract

Helicobacter pylori (*H. pylori*) is uniquely adapted to colonize the gastric mucosa. The key components for gastric colonization include motility, adhesion, and acid acclimation. The flagellar system allows the bacteria to move within the gastric mucus layer to the sites where conditions are optimal for survival. Adhesion to the gastric mucosa, via interaction between bacterial and host proteins, allows the bacteria to withstand the bulk flow of gastric fluid. Acid acclimation is the system that allows for periplasmic and cytoplasmic pH regulation in the setting of an acidic environment. The bacteria are bioenergetically neutralophiles, meaning they are able to survive between pH 4 and 8 and grow between pH 6 and 8. The pH at the gastric surface in the presence of *H. pylori*, as shown by microelectrode, fluorescent dye, and in vivo transcriptome studies, is below the range for growth and near to below the limits for survival. The bacteria are able to sense acidic medium pH and stimulate the trafficking of cytoplasmic urease and its accessory proteins to the proton-gated urea channel, UreI, in the inner membrane. The breakdown of urea into carbon dioxide and ammonia buffers the periplasm and cytoplasm to within the pH range optimal for a neutralophile. Understanding gastric colonization is clinically relevant because these systems that facilitate colonization can be targeted or interfered with to improve the efficacy of eradication regimens.

Keywords

Helicobacter pylori · Colonization · Acid acclimation · Adhesion · Motility

2.1 Introduction

Colonization of the host by a microbe refers to a state of infection resulting in a continuum of disease from none to significant. Colonization where no disease is present is synonymous with commensalism, as is seen with normal human gut flora. On the other side of the disease spectrum, bacteria that cause damage to the host are defined as pathogens. Therefore, colonization factors are distinct from pathogenic factors in that they are required for initial and persistent infection, but do not necessarily result in damage to the host. Pathogenic factors, on the other hand, are not required for initial and persistent infection but cause damage to the host. *Helicobacter pylori* (*H. pylori*) infection always results in chronic active gastritis, defining it as a pathogen, even though most infected individuals are asymptom-

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atic. This chapter describes the gastric environment that *H. pylori* colonizes, followed by a discussion of the colonization factors used by *H. pylori* to overcome the impediments to infection that make it uniquely suited to colonize and persist in the gastric milieu.

2.2 Gastric Environment at the Site of Infection

The human stomach, in the setting of normal physiologic acid secretion, has a median luminal pH of about 1.4, with elevations up to about pH 4 at mealtimes due to buffering by food [1]. It was originally believed that *H. pylori* inhabited a more neutral niche at the gastric surface with protection from gastric acidity provided by bicarbonate secretion from epithelial cells and by the mucus layer. Studies done with glass-tipped microelectrodes suggested a pH gradient through the gastric mucus, with pH near neutral at the epithelium [2]. These studies may have been hindered by measurement techniques as the open-tip microelectrodes may have prevented the diffusion of protons. Later microelectrode studies using a similar measurement system in mice suggested that the presence of *H. pylori* removed any barriers to proton diffusion and suggested an acidic pH at the bacterial niche [3]. Fluorescent dye studies done in the externalized stomachs of anesthetized mice again suggested an acidic gastric surface pH, regardless of the presence of the mucus layer [4]. The pH at the gastric surface is a combination of regulation of acid and alkali secretion to a specific set point rather than a result of trapping of buffers or protons under the mucus layer [4].

Transcriptome analysis of *H. pylori* provides further evidence for acidic pH at the gastric surface. Several in vitro studies have outlined changes in gene expression at acidic pH, using a variety of incubation times and conditions [5–7]. The unifying conclusion from these studies, done using divergent methodology, is that there is a set of genes that change expression based on environmental pH, suggesting adaptation to allow for gastric colonization. The well-documented movement of *H. pylori* from its typical niche in the gastric antrum to the fundus in the setting of acid-inhibitory therapy in both humans and gerbils again provides evidence for the need for the bacteria to inhabit a very specific environment with a specific pH range [8–11]. The *H. pylori* transcriptome has been studied in the gerbil stomach to correlate with in vitro pH changes [12]. The gerbil is a viable model system because the gastric pH profile and the advanced sequelae of *H. pylori* infection are similar to those seen in humans [13–15]. The pattern of *H. pylori* gene changes in the gerbil stomach was comparable to gene changes seen in vitro at acidic pH, providing additional evidence for an acidic environment at the site of infection [12].

2.3 Motility

H. pylori infection is acquired in early childhood through oral–oral or oral–fecal routes. In the first step toward colonization, the microbe must reach the mucosa of the gastric antrum, its preferred site of colonization. To reach its gastric niche, *H. pylori* has evolved a spiral shape and unipolar flagella to transit the mucus barrier overlying the gastric epithelial surface. Host factors orient the movement of *H. pylori* toward the gastric mucosa through the bacterial chemotactic response (Fig. 2.1).

The need for motility as a colonization factor was demonstrated using *H. pylori* strains with different degrees of motility. It was found that the gastric infection in gnotobiotic pigs was greater in the more motile strains of *H. pylori* [16]. Infection of gnotobiotic pigs by aflagellate mutants was cleared in 6 days, while the flagellate parent strain remained infective for at least 3 weeks [17]. The flagellar structure of *H. pylori* is similar to other bacterial flagella and is composed of two substructures, the hook-basal body (HBB), which drives flagellar movement, and the extracellular localized flagellum. The HBB has components located in the cytoplasm, the periplasm, and the inner and outer membranes. Flagellar gene expression is temporally regulated with transcription proceeding from intracellular

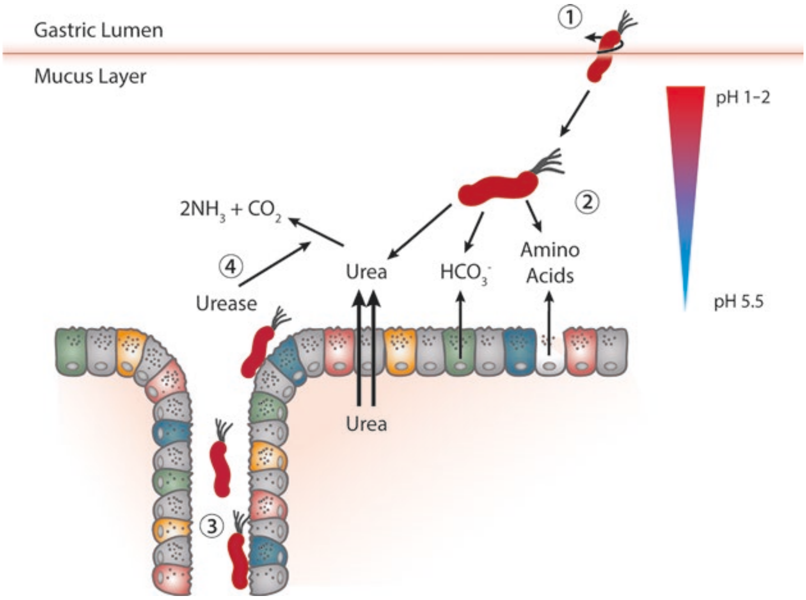

Fig. 2.1 Model of colonization of the gastric mucosa by *H. pylori* (*1*) The spiral shape and unipolar flagella propel *H. pylori* in a counterclockwise rotation to auger through the gastric mucus and reach the gastric epithelial surface, the site of colonization. (*2*) *H. pylori* is lured to the gastric surface by the chemoattractant, urea, which enters the gastric lumen both trans- and paracellularly, bicarbonate released from surface cells, and various amino acids released from damaged surface epithelial cells. (*3*) Upon reaching the gastric surface, the bacteria adhere to adhesin receptors on the cell surface. (*4*) Through the mechanism of acid acclimation, *H. pylori* are able to survive and thrive in a highly acidic environment

localized components to the extracellular flagellar filaments.

In the normal acid-secreting stomach, *H. pylori* is found predominately within the first 15–30 μm of the mucus overlying the antrum, with about one-third of the bacteria in the mucus layer immediately adjacent to the epithelial cells (0–5 μm) [18]. About 2% of the bacteria are found adhering to the gastric epithelium. To colonize this niche, the bacteria sense host attractants or repellents and move toward or away from them, respectively. Urea, bicarbonate, pH, zinc, nickel, arginine, glutamine, histidine, and other amino acids elicit chemotactic responses by *H. pylori* [19–23]. These chemotactic factors are sensed by methyl-accepting chemotaxis proteins (MCPs) that transduce the signal and alter flagellar rotation. *H. pylori* has at least four MCPs, the membrane proteins TlpA, TlpB, and TlpC, and the cytoplasm-located TlpD. TlpA senses arginine, other amino acids, and bicarbonate [19]. TlpB is required for pH and urea taxis and also senses the quorum-sensing molecule autoinducer-2 (AI-2) [24]. *H. pylori* gathers at sites of gastric injury and limits repair mechanisms, and TlpB is necessary for proper orientation of the bacteria at sites of attempted repair [25, 26]. AI-2 is produced by the bacteria and reduces biofilm formation [27]. TlpC regulates whether acid is sensed as an attractant or repellent [22]. TlpD senses the internal energy state of the bacterium [28]. TlpD senses trace concentrations of hypochlorous acid, likely from neutrophils, which may attract *H. pylori* to inflamed gastric glands at sites of injury, where they are able to persist [29].

2.4 Adhesion

Adherence of *H. pylori* to the gastric epithelium is a necessary step in establishing successful infection because it provides protection from clearance mechanisms such as bulk liquid flow, gastric peristalsis, and the continuous shedding and replenishment of the mucus layer (Fig. 2.1). *H. pylori* have evolved specific virulence determinates on their outer membrane which recognize distinct proteins, proteolipids, or carbohydrates expressed on epithelial cells. These virulence factors are adhesins, which bind to receptors on the surface of the gastric mucosa. Many of the adhesins are outer membrane proteins (OMPs). The histo-blood group antigen Lewis b (Le^b) was identified as an adhesin receptor for *H. pylori* as shown by antibody competition assays and the lack of binding to gastric epithelia not expressing Le^b [30]. Le^b is the dominant antigen in the human gastric mucosa of individuals that secrete α1, 2-fucosyltransferase [31]. The Le^b phenotype is epidemiologically associated with the presence of the *cag* PAI [32, 33]. The adhesin recognizing the Le^b antigen was isolated by affinity purification. This adhesin is an *H. pylori* OMP and was named BabA (blood group antigen-binding adhesin) [33]. *H. pylori* infection of the gastric mucosa results in chronic active gastritis. This inflammation results in the replacement of the naturally produced Lewis antigens and the expression of sialylated glycans such as sialyl Le^a and sialyl Le^x [34, 35]. In the absence of BabA, an adhesin was identified that binds to the sialyl Le^x antigen and named SabA (sialic acid-binding adhesin) [34]. *H. pylori* isogenic mutants lacking the OMP HopZ failed to adhere to the gastric carcinoma-derived AGS cells [36], indicating that it is an adhesin, but its receptor is unknown. HopZ transcription is regulated by pH change [6], indicating sensitivity to the bacterial environment. Two other OMPs, the adherence-associated lipoproteins A and B (AlpA and AlpB), have been identified as putative adhesins due to loss of binding to the gastric epithelium of their respective isogenic mutants [37]. As with HopZ, the host receptors have not been identified. The presence of HopZ and AlpA has been associated with gastric cancer development when compared to chronic gastritis development [38], providing further evidence for the importance of adhesion of *H. pylori* in gastric disease.

2.5 Acid Acclimation

Gastric acid is an impediment to colonization. *H. pylori* is a neutralophile that grows between pH 6.0 and 8.0 and survives between pH 4.0 and 8.0. Since the median luminal pH of the stomach is less than 2.0 and *H. pylori* not only survives in this high acidity but flourishes, it has evolved the unique mechanism of acid acclimation. Acid acclimation is the ability of *H. pylori* to maintain periplasmic pH near neutral in an acidic environment [39]. This is distinct from the acid resistance mechanisms that allow bacteria to transit the stomach by maintaining a cytoplasmic pH near 5 [40]. Examples of proteins involved with acid resistance include the glutamate decarboxylase-glutamate aminobutyrate antiporter and the arginine decarboxylase-arginine-agmatine antiporter, which consume protons and produce carbon dioxide, and the proton transporters including the F_1F_0 ATPase and the $Na^+/2H^+$ antiporter [41, 42]. These systems are designed to buffer the cytoplasm but do not regulate periplasmic pH. Gastric colonization is not possible if cytoplasmic pH cannot be elevated to a level that allows critical metabolic processes such as protein synthesis, a level of buffering that requires periplasmic pH regulation [39]. While *H. pylori* expresses some of the known acid resistance or tolerance genes [43], these proteins complement rather than explain gastric colonization.

The principal component of acid acclimation is the neutral pH optimum, highly expressed cytoplasmic urease enzyme. The *H. pylori* urease gene cluster is made up of seven genes under the control of two promoters [44]. *ureA* and *ureB*, under the control of the first promoter, encode the structural subunits of the urease enzyme [44]. Urease is a hexameric heterodimer that requires nickel incorporation for activation. Downstream from the second promoter are *ureI*, *ureE*, *ureF*, *ureG*, and *ureH* [44]. *ureI* encodes the only integral membrane protein in the operon. UreE, UreF, UreG, and UreH are cytoplasmic accessory proteins that aid in nickel incorporation into apourease.

Urease is required for acid survival and gastric colonization [45, 46]. The production of urease by *H. pylori* is constitutive and accounts for about 10% of the total cellular protein [47, 48]. The cytoplasmic enzyme with a neutral pH optimum catalyzes the breakdown of urea into carbonic acid and then to carbon dioxide and ammonia. The activity curve of free urease shows the expected pH optimum near neutral, with minimal activity at acidic pH and inactivation as the pH drops into the range found in the stomach. In contrast, in the intact bacteria, urease activity is minimal at neutral pH and rises to maximal below pH 6, down to about pH 2.5 [49]. This activity curve suggests a barrier of access of urea to the urease enzyme (Fig. 2.2). UreI, the only membrane protein in the urease gene cluster, was shown to be a proton-gated urea channel, allowing urea into the cytoplasm at acidic pH [50]. Deletion of *ureI* leads to loss of acid activation of urease [49]. At physiologic urea concentrations, *ureI* deletion mutants are unable to survive in acid. When medium pH drops, periplasmic pH drops as well. This leads to the opening of UreI, the movement of urea into the cytoplasm, and breakdown to the eventual end products of carbon dioxide and ammonia, catalyzed by the urease enzyme. These two gasses then buffer the periplasm into the pH range compatible with the survival of a neutralophile, without the need for bulk pH change in the environment (Fig. 2.3).

2 Ni^{2+} per active site is required for the activation of urease, and a large fraction of urease can be inactive, especially at neutral pH [51, 52]. Presumably, this would prevent over-alkalization of this neutralophile in conditions where the pH rises and would also create a pool of urease that is ready and available to be activated in the setting of a drop in pH. The cytoplasmic accessory proteins are critical to urease activation and activity [53]. UreE forms a heterodimer with UreG and UreF with UreH, as evidenced by yeast two-hybrid and homology analysis, and these protein pairs bind urease most likely via UreB to aid with nickel incorporation and enzyme activation [54,

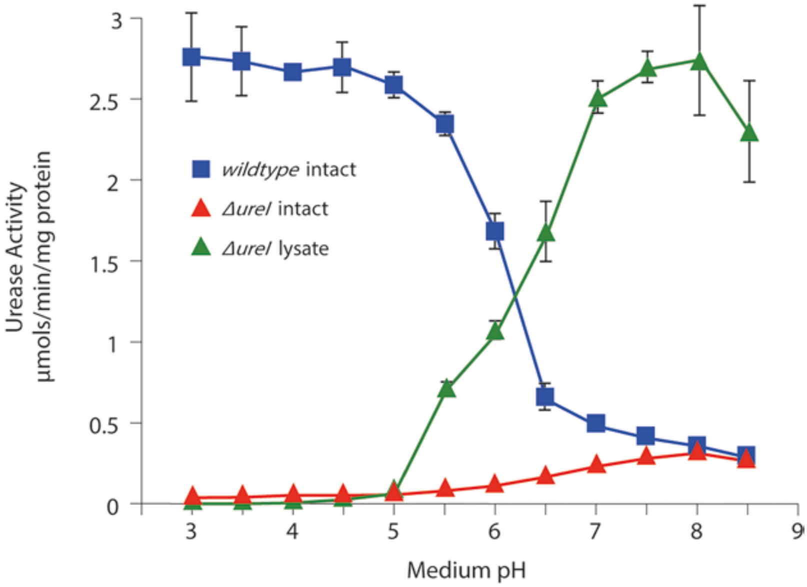

Fig. 2.2 *H. pylori* urease has a neutral pH optimum but is acid activated in intact bacteria. Activity of free urease enzyme, measured by the release of ^{14}C-labeled CO_2 from ^{14}C-labeled urea, peaks at near-neutral pH, and the enzyme is inactive below pH 4 (not shown). In intact bacteria, there is minimal activity at pH 7, when the proton-gated urea channel, UreI, is mainly closed, and activity increases as medium pH drops and urea gains access to cytoplasmic urease. In the absence of UreI, acid acclimation is not seen and activity is low at acidic medium pH. The activity of urease enzyme itself is unaffected by the absence of UreI, as evidenced by the normal-appearing activity curve in lysed bacteria

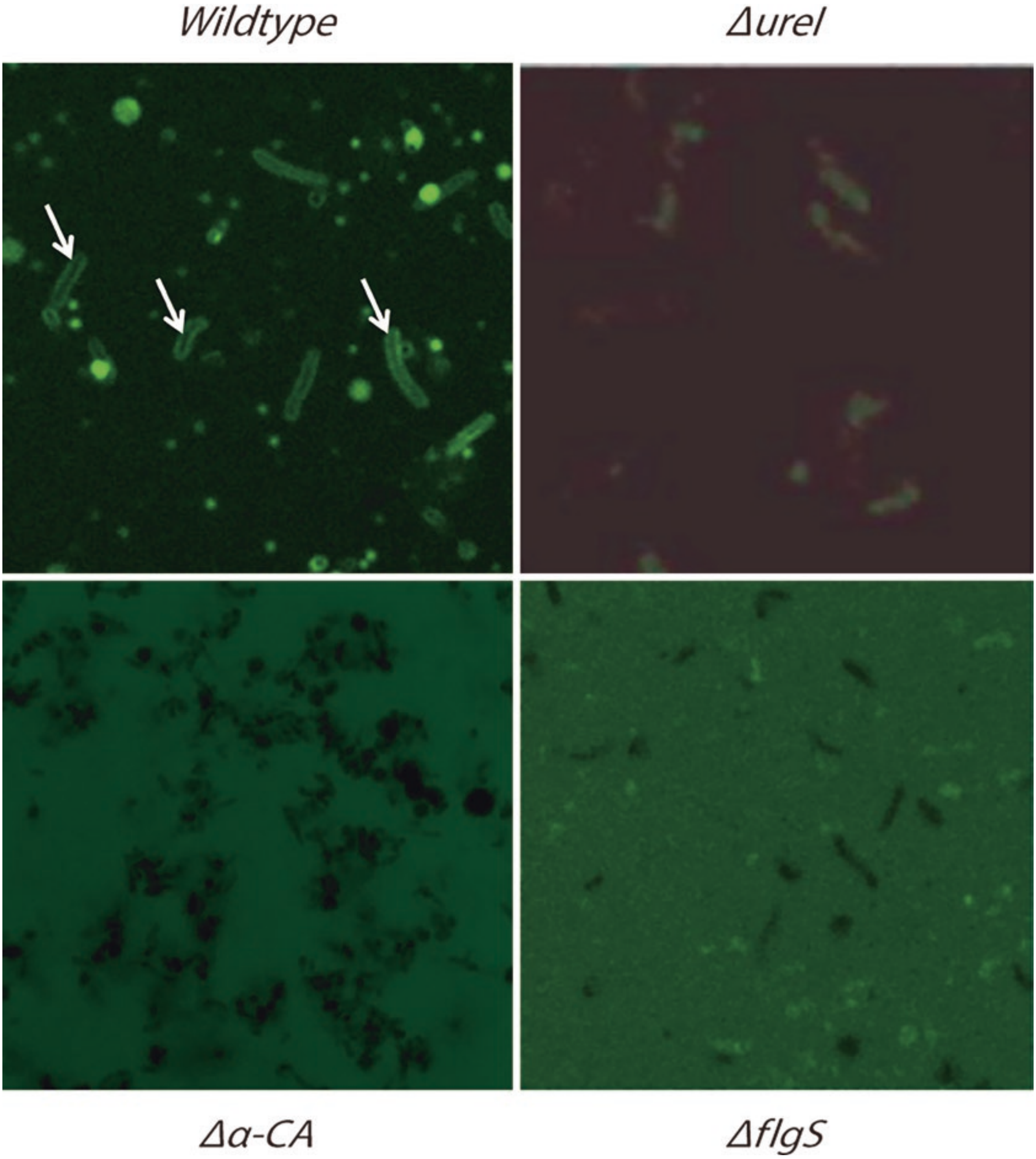

Fig. 2.3 Periplasmic alkalization is seen in *wild-type H. pylori* at an acidic medium pH in the presence of urea. The fluorescent, impermeant, pH-sensitive dye BCECF free acid was used to look at periplasmic pH. At acidic pH, in *wild-type* bacteria, periplasmic buffering facilitated by urease activity and the urea channel, UreI, is shown by an increase in periplasmic fluorescence with the addition of urea (*arrows*). In the absence of expression of key acid acclimation proteins, such as UreI, α-carbonic anhydrase (CA), and FlgS, periplasmic pH change is significantly reduced

55]. Each accessory protein has a specific role in urease activation. UreE aids directly with the incorporation of nickel into the active site [55]. UreF prevents premature nickel binding [56]. UreG provides energy for assembly of urease [57]. UreH provides stability for apourease [58].

The nickel regulatory protein NikR is able to directly and indirectly control a wide range of regulatory systems, many of which are involved in acid survival [59]. For example, NikR has been shown both in vitro and in vivo to positively regulate the expression of *ureA* [60–63].

While the urease operon and the regulation of nickel uptake are critical for gastric colonization, other proteins are also prominently involved in acid acclimation. *H. pylori* expresses a β-carbonic anhydrase enzyme in the cytoplasm that catalyzes the production of carbon dioxide from the intermediate products of the urease reaction. An α-carbonic anhydrase enzyme is localized to the inner membrane/periplasm and aids in the generation of bicarbonate from carbon dioxide entering the periplasm. While ammonia can neutralize protons in both the cytoplasm and periplasm, the pK_a of ammonia/ammonium is about 9.2, so the buffering capacity of this product of urea hydrolysis is limited at the effective pH seen by the bacteria. In contrast, the pK_a of carbon dioxide/bicarbonate is around 6.1, which is closer to the range needed for *H. pylori* growth and for urea channel opening. Carbon dioxide is therefore a major contributor to the periplasmic buffering unique to *H. pylori* acid acclimation [39]. Inhibition or deletion of the α-carbonic anhydrase limits *H. pylori* acid survival, and periplasmic buffering is not seen using fluorescent dye and confocal microscopy [39] (Fig. 2.3).

Efficiency of response to medium acidification is also critical to *H. pylori* acid acclimation. If periplasmic buffering does not occur rapidly, the cytoplasmic pH will drop and critical metabolic processes will be impaired. The structural subunits of the urease enzyme, UreA and UreB, interact with the urea channel, UreI, at the inner membrane, as demonstrated by native electrophoresis of purified membrane preparations, antibody isolation of the proteins, and electron microscopy [55, 64]. UreA and UreB are not found in purified membranes in the absence of UreI [55]. Urease is also activated, by incorporation of nickel, at the membrane. The products of urea hydrolysis, carbon dioxide, and ammonia, as gasses, are able to diffuse through the inner membrane to buffer the periplasm, and in addition, they are able to move back through UreI, which further enhances the efficiency of response [65].

Bacterial two-component systems (TCSs) allow bacteria to sense and respond to their environment. They are classically composed of a histidine kinase that senses changes in the environment and autophosphorylates and a response regulator that is activated by phosphotransfer to an aspartyl residue to regulate gene transcription [66]. *H. pylori* has relatively few TCSs compared with other bacteria, reflective of their environmental niche and the consistent environment in the stomach [43]. Two of these TCSs, ArsRS and FlgRS, are involved with the sensing of environmental pH (Figs. 2.4 and 2.5). The histidine kinase ArsS is localized to the inner membrane and is able to sense periplasmic pH via protonation of at least histidine 94 [67]. Both acid-responsive TCSs are involved with the trafficking of urease and the accessory proteins to the inner membrane in response to medium acidity [65, 68]. In the case of ArsRS, it has been demonstrated that this response is driven by the sensor kinase [69]. When medium pH drops, ArsS senses the drop in pH and stimulates the movement of urease to UreI at the inner membrane [68]. Accessory proteins required for nickel incorporation and urease activation also move to the inner membrane [68]. In the absence of ArsS, the levels of urease and the cytoplasmic accessory proteins in the membrane fraction and total urease activity of this fraction are reduced [68]. *ΔarsS* deletion mutants are defective in the colonization of the mouse stomach [70]. Proton gating of the urea channel, UreI, is unaffected by the absence of ArsS [68]. ArsR is essential for bacterial viability, but strains with a point mutation preventing phosphorylation are viable, and phosphorylation is not required for acid survival [69, 71]. ArsR phosphorylation is required for upregulation and transcriptional control of acid acclimation genes [72] and provides negative feedback on its own promoter to prevent overalkalization [73].

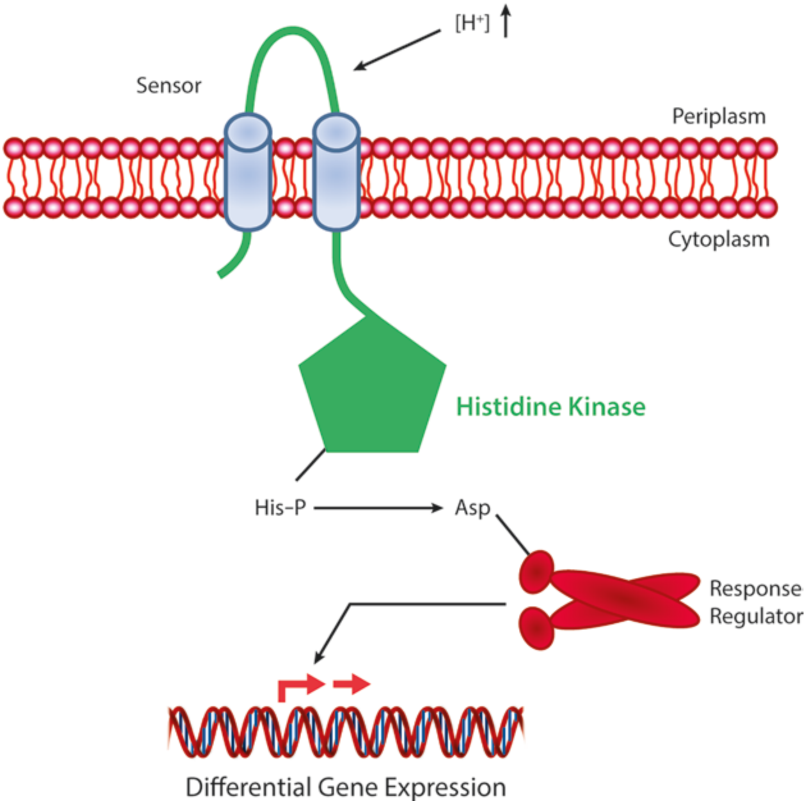

Fig. 2.4 Model of bacterial two-component systems (TCSs). *H. pylori* has two pH-sensitive TCSs. ArsRS responds to a drop in periplasmic pH and FlgRS to a drop in cytoplasmic pH. ArsRS is depicted in the model. The histidine kinase ArsS is in the membrane. When it senses a drop in pH, it is autophosphorylated, and then the phosphate is transferred to an asparagine residue on the cytoplasmic response regulator. The response regulator is then activated to regulate gene transcription, including the expression of acid acclimation genes

The histidine kinase FlgS is cytoplasmic and therefore senses cytoplasmic pH. FlgS is required for *H. pylori* viability at medium pH 2.5 in the presence of urea, independent of the response regulator FlgR [74]. In addition to pH-dependent genes, FlgRS also controls intermediate flagellar genes [74, 75]. FlgS, like ArsS, is involved with the trafficking of urease and its cytoplasmic accessory proteins to the plasma membrane [65]. Since it is cytoplasmic, FlgS is positioned to respond to lower media pH and the resultant lower cytoplasmic pH [7, 65, 74]. Urease activity over time at acidic pH in a *ΔarsS* deletion mutant, initially decreased, is able to recover by 90 min, presumably because the cytoplasmic pH drops far enough by this point to activate FlgS [68]. The absence of *flgS* leads to an inability of the bacteria to buffer periplasmic pH in acid and to the loss of membrane potential at extreme acidity in the presence of urea, both requirements for gastric colonization [65].

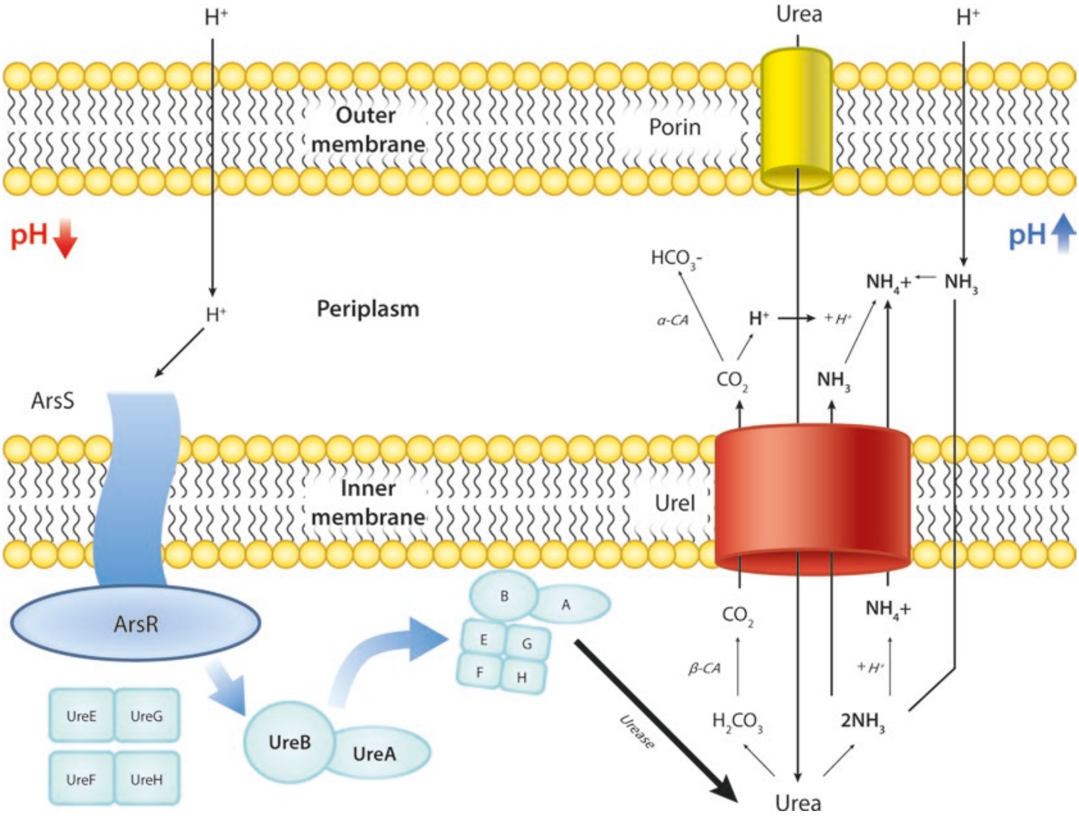

Fig. 2.5 Model of *H. pylori* acid acclimation. When medium pH drops, periplasmic pH drops as well. This leads to the opening of UreI, the proton-gated urea channel in the inner membrane. The urea moves into the cytoplasm. At the same time, the fall in pH is sensed by ArsS in the inner membrane and, with a further fall in pH, by FlgS in the cytoplasm (omitted for clarity). Activation of the TCSs leads to the trafficking of urease and its accessory proteins to the urea channel at the inner membrane. Urease is then activated at the membrane via nickel incorporation, leading to the breakdown of urea into carbonic acid and ammonia. Carbonic acid is converted to CO_2 with the help of the cytoplasmic β-carbonic anhydrase enzyme. The two gasses diffuse through the membrane and through the urea channel into the periplasm, where CO_2 is converted to bicarbonate with the help of the periplasmic α-carbonic anhydrase enzyme. The periplasm is then buffered to a pH within range for the survival of a neutralophile

2.6 pH Alteration and Treatment Efficacy

H. pylori is uniquely adapted to survive in the acidic gastric environment, but, as a neutralophile, the bacteria will divide and grow at neutral pH. There is an increase in transcription of growth-dependent genes at higher medium pH [76]. Many of the antibiotics used to treat *H. pylori* infection are dependent on bacterial growth for maximal efficacy. The action of ampicillin against *H. pylori* in vitro is significantly greater at near-neutral pH [76]. The addition of bismuth to treatment regimens also has, at least in part, a pH-based effect, as the compound impedes proton entry, leading to attenuation of the drop in cytoplasmic pH with medium acidification, allowing for increased bacterial metabolism and increased antibiotic efficacy [77]. With this in mind, the more bacteria that are dividing at the time of treatment, the more effective the treatment will be using standard, proton pump inhibitor, and antibiotic-based triple or quadruple therapy regimens. This concept is likely homologous to the concept of persisters seen across bacterial species. Persisters are members of a bacterial population that survive exposure to bactericidal antibiotics yet, when re-cultured, display the same antibiotic sensitivity as the population as a whole [78–80]. *H. pylori* that are not dividing at the time of antibiotic administration will not be killed by the antibiotics, leaving a small population of viable bacteria that can restore colonization of the stomach once the antibiotics are stopped. Medications currently available for acid blockade, at recommended doses, will not achieve the sustained pH change required to mimic the bactericidal effect seen in in vitro studies [76, 81]. Newer acid-suppressive medications offer hope for increased antibiotic efficacy. The potassium-competitive acid blocker vonoprazan was found in one meta-analysis to be superior to proton pump inhibitors for first-line *H. pylori* treatment [82]. The existing problems with treatment efficacy could also be overcome through the development of nonantibiotic treatment regimens that take advantage of the colonization mechanisms described here, via prevention of or interference with motility, adhesion, or acid acclimation. The in vitro efficacy of the carbonic anhydrase inhibitor acetazolamide against *H. pylori* is one example of a potential treatment targeting acid acclimation and periplasmic pH regulation [39]. Continued study and understanding of the molecular mechanisms of gastric colonization of *H. pylori* are critical for the development of new and improved treatment regimens.

2.7 Conclusions

The pathogenic bacterium *H. pylori* has evolved a series of mechanisms to ensure persistent infection of the gastric environment. Among these are the ability to sense and transit to its preferred site of colonization at cell–cell junctions of the antral mucosa. The bacterium recognizes and binds to host adhesin receptors to prevent shedding into the stomach lumen. To combat gastric acidity, *H. pylori* is uniquely able to maintain periplasmic pH near neutrality through the urease system to maintain the bioenergetic profile of a neutralophile. Knowledge of the mechanisms that allow gastric colonization is critical for the development of novel treatment regimens.

References

1. Teyssen S, Chari ST, Scheid J, Singer MV. Effect of repeated boluses of intravenous omeprazole and primed infusions of ranitidine on 24-hour intragastric pH in healthy human subjects. Dig Dis Sci. 1995;40:247–55.
2. Code CF. Defense mechanisms of the gastric mucosa. Scand J Gastroenterol Suppl. 1981;67:201–4.
3. Henriksnas J, Phillipson M, Storm M, Engstrand L, Soleimani M, Holm L. Impaired mucus-bicarbonate barrier in *Helicobacter pylori*-infected mice. Am J Physiol Gastrointest Liver Physiol. 2006;291:G396–403.
4. Baumgartner HK, Montrose MH. Regulated alkali secretion acts in tandem with unstirred layers to regulate mouse gastric surface pH. Gastroenterology. 2004;126:774–83.
5. McGowan CC, Necheva AS, Forsyth MH, Cover TL, Blaser MJ. Promoter analysis of *Helicobacter pylori* genes with enhanced expression at low pH. Mol Microbiol. 2003;48:1225–39.
6. Merrell DS, Goodrich ML, Otto G, Tompkins LS, Falkow S. pH-regulated gene expression of the gastric pathogen *Helicobacter pylori*. Infect Immun. 2003;71:3529–39.
7. Wen Y, Marcus EA, Matrubutham U, Gleeson MA, Scott DR, Sachs G. Acid-adaptive genes of *Helicobacter pylori*. Infect Immun. 2003;71:5921–39.
8. Larkin CJ, Watson RGP, Sloan JM, Stevenson M, Ardill JE, Buchanan D. Distribution of atrophy in *Helicobacter pylori*-infected subjects taking proton pump inhibitors. Scand J Gastroenterol. 2000;35:578–82.
9. Lee A, Dixon MF, Danon SJ, Kuipers E, Megraud F, Larsson H, et al. Local acid production and *Helicobacter pylori*: a unifying hypothesis of gastroduodenal disease. Eur J Gastroenterol Hepatol. 1995;7:461–5.
10. Logan RP, Walker MM, Misiewicz JJ, Gummett PA, Karim QN, Baron JH. Changes in the intragastric distribution of *Helicobacter pylori* during treatment with omeprazole. Gut. 1995;36:12–6.
11. Mollenhauer-Rektorschek M, Hanauer G, Sachs G, Melchers K. Expression of UreI is required for intragastric transit and colonization of gerbil gastric mucosa by *Helicobacter pylori*. Res Microbiol. 2002;153:659–66.

12. Scott DR, Marcus EA, Wen Y, Oh J, Sachs G. Gene expression *in vivo* shows that *Helicobacter pylori* colonizes an acidic niche on the gastric surface. Proc Natl Acad Sci U S A. 2007;104:7235–40.
13. Hirayama F, Takagi S, Kusuhara H, Iwao E, Yokoyama Y, Ikeda Y. Induction of gastric ulcer and intestinal metaplasia in Mongolian gerbils infected with *Helicobacter pylori*. J Gastroenterol. 1996;31:755–7.
14. Matsumoto S, Washizuka Y, Matsumoto Y, Tawara S, Ikeda F, Yokota Y, et al. Induction of ulceration and severe gastritis in Mongolian gerbil by *Helicobacter pylori* infection. J Med Microbiol. 1997;46:391–7.
15. Watanabe T, Tada M, Nagai H, Sasaki S, Nakao M. *Helicobacter pylori* infection induces gastric cancer in Mongolian gerbils. Gastroenterology. 1998;115:642–8.
16. Eaton KA, Morgan DR, Krakowka S. *Campylobacter pylori* virulence factors in gnotobiotic piglets. Infect Immun. 1989;57:1119–25.
17. Eaton KA, Morgan DR, Krakowka S. Motility as a factor in the colonisation of gnotobiotic piglets by *Helicobacter pylori*. J Med Microbiol. 1992;37:123–7.
18. Schreiber S, Konradt M, Groll C, Scheid P, Hanauer G, Werling HO, et al. The spatial orientation of *Helicobacter pylori* in the gastric mucus. Proc Natl Acad Sci U S A. 2004;101:5024–9.
19. Cerda O, Rivas A, Toledo H. *Helicobacter pylori* strain ATCC700392 encodes a methyl-accepting chemotaxis receptor protein (MCP) for arginine and sodium bicarbonate. FEMS Microbiol Lett. 2003;224:175–81.
20. Foynes S, Dorrell N, Ward SJ, Stabler RA, McColm AA, Rycroft AN, et al. *Helicobacter pylori* possesses two CheY response regulators and a histidine kinase sensor, CheA, which are essential for chemotaxis and colonization of the gastric mucosa. Infect Immun. 2000;68:2016–23.
21. Nakamura H, Yoshiyama H, Takeuchi H, Mizote T, Okita K, Nakazawa T. Urease plays an important role in the chemotactic motility of *Helicobacter pylori* in a viscous environment. Infect Immun. 1998;66:4832–7.
22. Sanders L, Andermann TM, Ottemann KM. A supplemented soft agar chemotaxis assay demonstrates the *Helicobacter pylori* chemotactic response to zinc and nickel. Microbiology. 2013;159(Pt 1):46–57.
23. Worku ML, Karim QN, Spencer J, Sidebotham RL. Chemotactic response of *Helicobacter pylori* to human plasma and bile. J Med Microbiol. 2004;53(Pt 8):807–11.
24. Rader BA, Wreden C, Hicks KG, Sweeney EG, Ottemann KM, Guillemin K. *Helicobacter pylori* perceives the quorum-sensing molecule AI-2 as a chemorepellent via the chemoreceptor TlpB. Microbiology. 2011;157(Pt 9):2445–55.
25. Aihara E, Medina-Candelaria NM, Hanyu H, Matthis AL, Engevik KA, Gurniak CB, et al. Cell injury triggers actin polymerization to initiate epithelial restitution. J Cell Sci. 2018;131:jcs216317.
26. Hanyu H, Engevik KA, Matthis AL, Ottemann KM, Montrose MH, Aihara E. *Helicobacter pylori* uses the TlpB receptor to sense sites of gastric injury. Infect Immun. 2019;87:e00202–19.
27. Anderson JK, Huang JY, Wreden C, Sweeney EG, Goers J, Remington SJ, et al. Chemorepulsion from the quorum signal autoinducer-2 promotes *Helicobacter pylori* biofilm dispersal. mBio. 2015;6:e00379.
28. Schweinitzer T, Mizote T, Ishikawa N, Dudnik A, Inatsu S, Schreiber S, et al. Functional characterization and mutagenesis of the proposed behavioral sensor TlpD of *Helicobacter pylori*. J Bacteriol. 2008;190:3244–55.
29. Perkins A, Tudorica DA, Amieva MR, Remington SJ, Guillemin K. *Helicobacter pylori* senses bleach (HOCl) as a chemoattractant using a cytosolic chemoreceptor. PLoS Biol. 2019;17:e3000395.
30. Boren T, Falk P, Roth KA, Larson G, Normark S. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science. 1993;262:1892–5.
31. Sakamoto S, Watanabe T, Tokumaru T, Takagi H, Nakazato H, Lloyd KO. Expression of Lewisa, Lewisb, Lewisx, Lewisy, siayl-Lewisa, and sialyl-Lewisx blood group antigens in human gastric carcinoma and in normal gastric tissue. Cancer Res. 1989;49:745–52.
32. Gerhard M, Lehn N, Neumayer N, Boren T, Rad R, Schepp W, et al. Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen-binding adhesin. Proc Natl Acad Sci U S A. 1999;96:12778–83.
33. Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, et al. *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science. 1998;279:373–7.
34. Mahdavi J, Sonden B, Hurtig M, Olfat FO, Forsberg L, Roche N, et al. *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. Science. 2002;297:573–8.
35. Ota H, Nakayama J, Momose M, Hayama M, Akamatsu T, Katsuyama T, et al. *Helicobacter pylori* infection produces reversible glycosylation changes to gastric mucins. Virchows Arch. 1998;433:419–26.
36. Peck B, Ortkamp M, Diehl KD, Hundt E, Knapp B. Conservation, localization and expression of HopZ, a protein involved in adhesion of *Helicobacter pylori*. Nucleic Acids Res. 1999;27:3325–33.
37. Odenbreit S, Faller G, Haas R. Role of the alpAB proteins and lipopolysaccharide in adhesion of *Helicobacter pylori* to human gastric tissue. Int J Med Microbiol. 2002;292:247–56.
38. Lee DH, Ha JH, Shin JI, Kim KM, Choi JG, Park S, et al. Increased risk of severe gastric symptoms by virulence factors vacAs1c, alpA, babA2, and hopZ in *Helicobacter pylori* infection. J Microbiol Biotechnol. 2021;31:368–79.
39. Marcus EA, Moshfegh AP, Sachs G, Scott DR. The periplasmic alpha-carbonic anhydrase activity of *Helicobacter pylori* is essential for acid acclimation. J Bacteriol. 2005;187:729–38.
40. Ma Z, Gong S, Richard H, Tucker DL, Conway T, Foster JW. GadE (YhiE) activates gluta-

mate decarboxylase-dependent acid resistance in *Escherichia coli* K-12. Mol Microbiol. 2003;49:1309–20.

41. Booth IR. Regulation of cytoplasmic pH in bacteria. Microbiol Rev. 1985;49:359–78.
42. Padan E, Zilberstein D, Schuldiner S. pH homeostasis in bacteria. Biochim Biophys Acta. 1981;650:151–66.
43. Tomb JF, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, et al. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature. 1997;388:539–47.
44. Akada JK, Shirai M, Takeuchi H, Tsuda M, Nakazawa T. Identification of the urease operon in *Helicobacter pylori* and its control by mRNA decay in response to pH. Mol Microbiol. 2000;36:1071–84.
45. Eaton KA, Brooks CL, Morgan DR, Krakowka S. Essential role of urease in pathogenesis of gastritis induced by *Helicobacter pylori* in gnotobiotic piglets. Infect Immun. 1991;59:2470–5.
46. Eaton KA, Krakowka S. Effect of gastric pH on urease-dependent colonization of gnotobiotic piglets by *Helicobacter pylori*. Infect Immun. 1994;62:3604–7.
47. Hu LT, Mobley HL. Purification and N-terminal analysis of urease from *Helicobacter pylori*. Infect Immun. 1990;58:992–8.
48. Mobley HL, Hu LT, Foxal PA. *Helicobacter pylori* urease: properties and role in pathogenesis. Scand J Gastroenterol Suppl. 1991;187:39–46.
49. Scott DR, Marcus EA, Weeks DL, Lee A, Melchers K, Sachs G. Expression of the *Helicobacter pylori ureI* gene is required for acidic pH activation of cytoplasmic urease. Infect Immun. 2000;68:470–7.
50. Weeks DL, Eskandari S, Scott DR, Sachs G. A H+-gated urea channel: the link between *Helicobacter pylori* urease and gastric colonization. Science. 2000;287:482–5.
51. Ha NC, Oh ST, Sung JY, Cha KA, Lee MH, Oh BH. Supramolecular assembly and acid resistance of *Helicobacter pylori* urease. Nat Struct Biol. 2001;8:505–9.
52. Scott DR, Marcus EA, Weeks DL, Sachs G. Mechanisms of acid resistance due to the urease system of *Helicobacter pylori*. Gastroenterology. 2002;123:187–95.
53. Ferrero RL, Cussac V, Courcoux P, Labigne A. Construction of isogenic urease-negative mutants of *Helicobacter pylori* by allelic exchange. J Bacteriol. 1992;174:4212–7.
54. Heimer SR, Mobley HL. Interaction of *Proteus mirabilis* urease apoenzyme and accessory proteins identified with yeast two-hybrid technology. J Bacteriol. 2001;183:1423–33.
55. Voland P, Weeks DL, Marcus EA, Prinz C, Sachs G, Scott D. Interactions among the seven *Helicobacter pylori* proteins encoded by the urease gene cluster. Am J Physiol Gastrointest Liver Physiol. 2003;284:G96–G106.
56. Moncrief MB, Hausinger RP. Purification and activation properties of UreD-UreF-urease apoprotein complexes. J Bacteriol. 1996;178:5417–21.
57. Moncrief MB, Hausinger RP. Characterization of UreG, identification of a UreD-UreF-UreG complex, and evidence suggesting that a nucleotide-binding site in UreG is required for *in vivo* metallocenter assembly of *Klebsiella aerogenes* urease. J Bacteriol. 1997;179:4081–6.
58. Park IS, Carr MB, Hausinger RP. In vitro activation of urease apoprotein and role of UreD as a chaperone required for nickel metallocenter assembly. Proc Natl Acad Sci U S A. 1994;91:3233–7.
59. van Vliet AH, Ernst FD, Kusters JG. NikR-mediated regulation of *Helicobacter pylori* acid adaptation. Trends Microbiol. 2004;12:489–94.
60. Carpenter BM, West AL, Gancz H, Servetas SL, Pich OQ, Gilbreath JJ, et al. Crosstalk between the *Hp*ArsRS two-component system and *Hp*NikR is necessary for maximal activation of urease transcription. Front Microbiol. 2015;6:558.
61. Dosanjh NS, Hammerbacher NA, Michel SL. Characterization of the *Helicobacter pylori* NikR-*P*(*ureA*) DNA interaction: metal ion requirements and sequence specificity. Biochemistry. 2007;46:2520–9.
62. Dosanjh NS, West AL, Michel SL. *Helicobacter pylori* NikR's interaction with DNA: a two-tiered mode of recognition. Biochemistry. 2009;48:527–36.
63. Evans SE, Michel SL. Dissecting the role of DNA sequence in *Helicobacter pylori* NikR/DNA recognition. Dalton Trans. 2012;41:7946–51.
64. Hong W, Sano K, Morimatsu S, Scott DR, Weeks DL, Sachs G, et al. Medium pH-dependent redistribution of the urease of *Helicobacter pylori*. J Med Microbiol. 2003;52(Pt 3):211–6.
65. Scott DR, Marcus EA, Wen Y, Singh S, Feng J, Sachs G. Cytoplasmic histidine kinase (HP0244)-regulated assembly of urease with UreI, a channel for urea and its metabolites, CO_2, NH_3, and $NH_4^{(+)}$, is necessary for acid survival of *Helicobacter pylori*. J Bacteriol. 2010;192:94–103.
66. Hoch JA. Two-component and phosphorelay signal transduction. Curr Opin Microbiol. 2000;3:165–70.
67. Muller S, Gotz M, Beier D. Histidine residue 94 is involved in pH sensing by histidine kinase ArsS of *Helicobacter pylori*. PLoS One. 2009;4:e6930.
68. Marcus EA, Sachs G, Wen Y, Feng J, Scott DR. Role of the *Helicobacter pylori* sensor kinase ArsS in protein trafficking and acid acclimation. J Bacteriol. 2012;194:5545–51.
69. Marcus EA, Sachs G, Wen Y, Scott DR. Phosphorylation-dependent and phosphorylation-independent regulation of *Helicobacter pylori* acid acclimation by the ArsRS two-component system. Helicobacter. 2016;21:69–81.
70. Loh JT, Cover TL. Requirement of histidine kinases HP0165 and HP1364 for acid resistance in *Helicobacter pylori*. Infect Immun. 2006;74:3052–9.
71. Forsyth MH, Cao P, Garcia PP, Hall JD, Cover TL. Genome-wide transcriptional profiling in a histidine kinase mutant of *Helicobacter pylori* identifies members of a regulon. J Bacteriol. 2002;184:4630–5.

72. Pflock M, Finsterer N, Joseph B, Mollenkopf H, Meyer TF, Beier D. Characterization of the ArsRS regulon of *Helicobacter pylori*, involved in acid adaptation. J Bacteriol. 2006;188:3449–62.
73. Dietz P, Gerlach G, Beier D. Identification of target genes regulated by the two-component system HP166-HP165 of *Helicobacter pylori*. J Bacteriol. 2002;184:350–62.
74. Wen Y, Feng J, Scott DR, Marcus EA, Sachs G. The pH-responsive regulon of HP0244 (*flgS*), the cytoplasmic histidine kinase of *Helicobacter pylori*. J Bacteriol. 2009;191:449–60.
75. Niehus E, Ye F, Suerbaum S, Josenhans C. Growth phase-dependent and differential transcriptional control of flagellar genes in *Helicobacter pylori*. Microbiology. 2002;148(Pt 12):3827–37.
76. Marcus EA, Inatomi N, Nagami GT, Sachs G, Scott DR. The effects of varying acidity on *Helicobacter pylori* growth and the bactericidal efficacy of ampicillin. Aliment Pharmacol Ther. 2012;36:972–9.
77. Marcus EA, Sachs G, Scott DR. Colloidal bismuth subcitrate impedes proton entry into *Helicobacter pylori* and increases the efficacy of growth-dependent antibiotics. Aliment Pharmacol Ther. 2015;42:922–33.
78. Amato SM, Fazen CH, Henry TC, Mok WW, Orman MA, Sandvik EL, et al. The role of metabolism in bacterial persistence. Front Microbiol. 2014;5:70.
79. Levin BR, Concepcion-Acevedo J, Udekwu KI. Persistence: a copacetic and parsimonious hypothesis for the existence of non-inherited resistance to antibiotics. Curr Opin Microbiol. 2014;21:18–21.
80. Lewis K. Persister cells. Annu Rev Microbiol. 2010;64:357–72.
81. Sugimoto M, Shirai N, Nishino M, Kodaira C, Uotani T, Yamade M, et al. Rabeprazole 10 mg q.d.s. decreases 24-h intragastric acidity significantly more than rabeprazole 20 mg b.d. or 40 mg o.m., overcoming CYP2C19 genotype. Aliment Pharmacol Ther. 2012;36:627–34.
82. Simadibrata DM, Syam AF, Lee YY. A comparison of efficacy and safety of potassium-competitive acid blocker and proton pump inhibitor in gastric acid-related diseases: a systematic review and meta-analysis. J Gastroenterol Hepatol. 2022;37:2217–28.

Immunological Reactions on *H. pylori* Infection

3

Nayoung Kim

Abstract

An immune network that responds to bacteria as a mucous membrane defense factor is called gut-associated lymphoid tissue (GALT), which is made up of distinctive tissues of B cells, T cells, and phagocytes. The purpose of GALT is to maintain stomach homeostasis via the interaction between immune and nonimmune mechanisms. Until 10–20 years ago, the stomach immune mechanism was mostly about *Helicobacter pylori* (*H. pylori*). However, recently non-*H. pylori* microbiota became very important. Thus, the crosstalk between *H. pylori* and gastrointestinal commensal flora may play a role in *H. pylori*–related carcinogenicity and extragastric manifestations. Anyway, long-term *H. pylori* proliferation indicates failure of gastric immune mechanisms that cause chronic *H. pylori* inflammation, and various diseases emerge as a result. *H. pylori* delicately regulates innate and adaptive immunities to evade host immunity. In particular, *H. pylori* disrupts T-cell activity such as T helper (Th)1/Th2 immunological reaction balance. Th1 produces cytokines like interferon-γ and interleukin (IL)-2, and Th2 produces cytokines like IL-4, IL-5, IL-10, and IL-13 and engages with the differentiation and activation of B cells. Finally, it causes gastritis, peptic ulcer, and mucosa-associated lymphoid tissue (MALT)-associated B call lymphoma and increases the risk of gastric cancer. Recently, the role of tight junction became important in this immune mechanism because *H. pylori* preferentially binds in close proximity to the tight junction and disrupts gastric barrier function, cell adhesion, and cell polarity, which culminates in an invasive phenotype.

Keywords

Immunity · Gut-associated lymphoid tissue
T helper cell · *Helicobacter pylori*

3.1 Introduction

The human stomach is constantly exposed to external antigens from food, gastric acid, and pepsin, so the stomach performs distinct and complex immune and non-immunological reaction mechanisms. Non-immunological reaction factors are mucus, epithelial cell phospholipid defense membrane, mucous membrane microcirculation, and nerve, immunity, and inflammatory mediators of mucous membrane recovery mechanism. These factors are directly related to ulcer or

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erosion recovery. An immune network that responds to bacteria as a mucous membrane defense factor is called gut-associated lymphoid tissue (GALT), which is made up of distinctive tissues of B cells, T cells, and phagocytes. GALT utilizes a special epithelium called follicle-associated epithelia (FAE) to gather antigens from the lumen and manages the functions of immune cells and nonimmune mucosal barrier compositions in a balanced condition. In other words, immune and nonimmune mechanisms have a close interaction to maintain stomach homeostasis.

While immune mechanisms in small and large intestines are well known due to their coexistence with bacteria, a gastric immune mechanism is mostly about *Helicobacter pylori* (*H. pylori*), since gastric acid makes a difficult condition for a bacterial invasion, whereas *H. pylori* can survive. A disease occurrence due to long-term *H. pylori* proliferation indicates the failure of gastric immune mechanisms and chronic *H. pylori* inflammation means the failure of appropriate activity by host immunity. The results of these clinical features due to chronic *H. pylori* infection are asymptomatic gastritis, mucosa-associated lymphoid tissue (MALT), gastric cancer (GC), and so on. These results are considered as the aftermaths of interactions between mucous membrane damage and defense mechanisms by immunological reactions on stomach epithelium, along with *H. pylori* toxin factors, host susceptibility, and other environmental factors. Recently, it has been known that *H. pylori* colonization significantly affects the gastric microenvironment, which, in turn, affects gastric microbiota and possibly colonic microbiota changes. Crosstalk between *H. pylori* and gastrointestinal (GI) commensal flora may play a role in *H. pylori*–related carcinogenicity and extragastric manifestations [1]. In addition, the role of tight junction became important in this immune mechanism because *H. pylori* or other non-*H. pylori* microbiota preferentially bind in close proximity to the tight junction and disrupt gastric barrier function, cell adhesion, and cell polarity, which culminates in an invasive phenotype. This chapter will discuss on general functioning of the gastric immune reaction and how immunological reactions for *H. pylori* invasion are different from normal immunological reactions, especially in terms of immune evasion [2].

3.2 Microbiota and General Immune Mechanism in the Stomach

The human gut, populated by complex communities of microorganisms, plays central roles in the digestion and absorption of nutrients [3], the stimulation of intestinal epithelial renewal [4], and immune responses [5]. Keeping these communities in balance with the host is crucial for health maintenance and disease prevention [6]. The human stomach as an ecological niche for bacteria received attention after the discovery of *H. pylori* in the 1980s. Before the discovery of *H. pylori*, the human stomach environment was thought to be sterile due to acidic conditions of pH <4, peristalsis, and high bile concentration suppressing the microorganisms from the oral cavity. Recent developments in molecular methods provided more detailed insights into the human microbiota in various organs including the skin, gut, and so on [7–10]. Analysis of the 16S ribosomal RNA (rRNA) gene contents of microbial samples after amplification by polymerase chain reaction (PCR) has revolutionized the characterization of microbial communities not only *H. pylori* but also other microbiota.

3.2.1 Microbiota in the Stomach and Their Possible Role

Although the stomach, along with the esophagus and the duodenum, is the least colonized region of the GI tract, in contrast to the high bacterial counts (10^{10}–10^{12} CFU/g) in the colon, the stomach also supports a bacterial community with hundreds of phylotypes [11–13]. While it has been postulated that the indigenous stomach microbiota might be a reflection of transient bacteria from the mouth and esophagus, microbiota were distinguishable from microbiota found in the mouth, nose, and distal GI tract [14]. The most abundant phyla were Proteobacteria, Firmicutes, and Actinobacteria in *H. pylori*-positive subjects and were Firmicutes, Bacteroidetes, and Actinobacteria in the absence of *H. pylori* [14]. In contrast, five major phyla were present in the majority of the samples in Kim's study: Proteobacteria, 65.0%; Firmicutes, 20.0%; Actinobacteria, 7.8%; Bacteroidetes, 4.0%; and Fusobacteria, 0.8% [15]. In addition, Proteobacteria, the phylum to which *H. pylori* belongs, was the major phylum in the gastric mucosa of both *H. pylori*-positive and *H. pylori*-negative subjects: *H. pylori*-negative control, 60.3%; *H. pylori*-negative GC, 50.9%; *H. pylori*-positive control, 82.4 %; and *H. pylori*-positive GC, 73.0 % [15]. The mean proportion of Proteobacteria was lower in the GC group than that in the control group, and the mean proportion of Actinobacteria was higher in the *H. pylori*-negative GC group, but they did not reach statistical significance [15].

In the GI tract, the microbiota has a major impact on the functioning of the mucosal immune system and vice versa. That is, germ-free mice have small size of Peyer's patches (PPs), decreased number of lamina propria immunoglobulin (Ig) A-secreting plasmocytes, and low levels of serum Ig and demonstrate no T helper (Th)17/Th1 in the intestine [15, 16]. The composition of the intestinal flora modulates the functioning of the immune system; for instance, the presence of segmented filamentous bacteria (SFB) in the microbiota is associated with the development of Th17 in the intestinal lamina propria [17]. The presence of some *Clostridia* strains within the human intestinal microbiota has been recently associated with the development of intestinal T-regulatory (Treg) cells [18]. In addition, some commensal bacteria and microbiota-derived metabolites like short-chain fatty acids have been shown to inhibit inflammatory reactions at intestinal levels and promote pathogen clearance [19–21]. Inversely, defects in antibody response lead to a modification of the bacterial composition of the intestinal flora [22].

The GI microbiota and the host generate a complex network of interactions that transcends the boundaries of the GI tract, forging intimate connections with all aspects of human physiology, including metabolic, immune, and neuroendocrine systems [1]. This crosstalk could be direct in the case of the local system but mostly mediated by microbial-derived biochemical signals that are absorbed into the blood and circulated throughout the human body; by signals relayed by the enteric nervous system that transmit microbiota-derived cues to the central nervous system; and by immune cells that perceive local microbial signals in the GI tract and are trafficked throughout the body [23–25].

3.2.2 General Immune Mechanism of Stomach

The immunity of the GI tract consists of innate and adaptive immunity. Innate immunity does not require acquired immunostimulation, and it is responsible for bacterial invasion as a first-line defense mechanism. A typical parameter of innate immunity is Toll-like receptor (TLR), which is a cluster of one of the embryologically preserved eukaryotic cell receptors that are made of transmembrane proteins. This receptor engages with innate immunity by perceiving the pathogenicity or molecular behavior of bacteria, and the receptor is related to the plasma membrane, ribosomal vesicle, or endosomal vesicle of stomach epithelium [26]. Eleven subtypes of TLR can be found among mammals, and the signal transduction via TLR ultimately activates pro-inflammatory genes via nuclear factor (NF)-κB activation [27]. Moreover, another important factor of innate immunity is nucleotide-binding oligomerization domain protein I (NodI) that exists in the cytoplasm. NodI detects peptidoglycans that are detached from cells and then induces epithelial cells to eliminate bacteria by themselves [26]. Adaptive immunity indicates a reaction on a previously known immunological stimulation that is specialized on a specific pathogen and depends on immunological memory. However, adaptive immunity shares similar characteristics with innate immunity, since lymphocytes are activated and recruited by the stimulation of macrophages and dendritic cells (DCs), and derives specific reactions of Th cells. As *H. pylori* colonizes the mucosal surface of the stomach, mucosal defenses are very important, which are physical, chemical, and immune-mediated [16]. The mucosal epithelium blocks invasion by pathogenic and commensal bacteria by forming multiple layers of physical (tight) junctions, chemical nitric oxide, and immune protection (local secretion of defensins, anti- and/or pro-inflammatory chemokines/cytokines, and IgA/IgG/IgM transport) [16]. In addition, numerous bone marrow-derived cells belonging to the innate or adaptive immune systems colonized the intestinal mucosa to fight the invaders, but at steady state, the same cells have to tolerate commensals [16]. Here, the epithelial defense mechanism of the GI tract will be described experientially.

3.2.2.1 IgA and IgG Response of Stomach

A major defensive mechanism that excludes commensals and pathogens from the mucosal surface involves IgA [28]. Mucosal IgA comprises antibodies that recognize antigens with high- and low-affinity binding modes. In general, high-affinity IgA neutralizes microbial toxins and invasive pathogens, whereas low-affinity IgA confines commensals in the intestinal lumen. High-affinity IgA is thought to emerge in PPs and mesenteric lymph nodes (MLNs) from follicular B cells stimulated via T cell-dependent pathways, whereas low-affinity IgA likely emerges in PPs, MLNs, and lamina propria from B cells stimulated via T cell-independent pathways [28]. IgA response is powerfully induced by the presence of commensal microbes in the intestine [29, 30] and has been shown to promote the maintenance of appropriate bacterial communities in specific intestinal segments [22]. In contrast to the lungs, vagina, and most of the GI tract, the healthy mammalian stomach produces very low levels of polymeric immunoglobulin receptor (pIgR) [31, 32], the receptor mediating IgA transport into the GI lumen. Studies in *H. pylori*-infected humans have shown that baseline pIgR expression by the gastric epithelium can be upregulated in response to gastric inflammation [33] due to increased local interferon (IFN)-γ production [34]. However, despite significantly increased pIgR expression and IgA plasma cell infiltration in response to *H. pylori* infection [35], there is no concomitant increase in IgA secretion into the stomach, and it is nonsecretory monomeric IgA that predominates in the stomach of *H. pylori*-infected individuals [36]. Hence, the IgA that is present in the gastric lumen would be unstable and susceptible to degradation by proteases. These observations suggest that the anti-*H. pylori* IgA responses in stomach do not play similar biological roles as compared with anti-commensal or anti-pathogen IgA responses taking place in the intestine. In contrast to gastric IgA response, systemic IgG response to gastric commensal is rather weak. That is, in unmanipulated specific pathogen-free animals, it has been shown that there was no specific serum IgG response detectable directed against commensal bacteria [37]. In pathogen-free mice, the systemic immune system appeared to remain ignorant of the commensal microbes. However, in humans, a certain degree of systemic exposure to gut commensal bacteria and the associated priming of systemic immune response seem to be well tolerated, harmless, and common in healthy humans since systemic antibody responses against live gut commensal bacteria and fungi can be detected [38]. Most of the *H. pylori*-infected individuals develop systemic anti-*H. pylori* IgG responses [39]. However, in the case of children, these anti-*H. pylori* IgG responses could be weak so that a serum antibody test is not recommended for the test of *H. pylori* infection.

3.2.2.2 CD4$^+$ T-Cell Responses

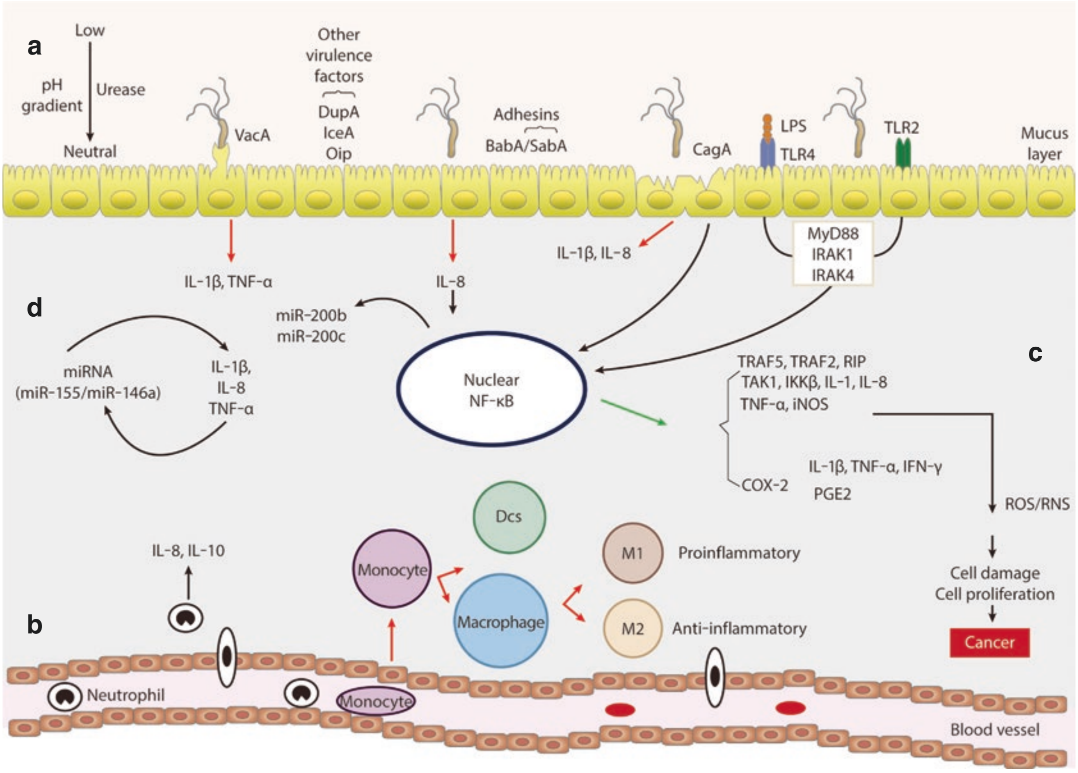
Since *H. pylori* is an extracellular bacteria, anti-*H. pylori*-specific CD8$^+$ T-cell responses are inadequate to protect the host against such pathogen, but priming of CD4$^+$ T-cell response is important [16]. CD4$^+$ T-cell responses are initiated within the PPs and MLNs. DCs capture, process, and present antigens to naive T cells in PPs and MLNs [16]. In the stomach, DCs are penetrating the mucosa [40] to sample luminal antigens and migrate to the stomach lymph node [41]. At a steady state, mucosal CD4$^+$ T cells are tolerant to microbiota-derived antigens [42]. Remarkably, systemic CD4$^+$ T cells are not tolerant to microbiota-derived antigens and conserved a naive state to these antigens [43]. Interestingly, antigen-specific intestinal IgA played a critical role in inhibiting the systemic CD4$^+$ T-cell responses to commensal antigens by providing immune exclusion [42]. At mucosal surfaces, DCs maintain homeostasis by dampening inflammatory Th1 and Th17 cell responses [44] because they receive conditioning signals from intestinal epithelial cells (IECs) [45, 46]. One of these signals is provided by thymic stromal lymphopoietin (TSLP), which shifts the Th1/Th2 balance toward Th2 polarization by attenuating DC production of interleukin (IL)-12 but not of IL-10 [47]. In addition to TSLP, IECs release transforming growth factor (TGF)-β and retinoic acid, which stimulate the development of CD103$^+$ DCs [44]. These DCs promote the formation of Treg cells via TGF-β and retinoic acid and suppress the development of inflammatory Th1 and Th17 cells [44]. In addition to initiating responses that create an overall tolerant state toward harmless intestinal antigens, mucosal DCs are also implicated in the generation of protective immune responses aimed at the clearance of enteric pathogens [16]. A fundamental difference between the steady state and a state of infection may lie in the greater propensity of pathogens to invade and penetrate beneath the epithelial cell layer [16]. Invasion of IECs would allow for the activation of cytosolic pattern recognition receptors, TLRs, and both quantitative and qualitative changes in the secretion of pro-inflammatory cytokines and chemokines. Consistent with this, IECs produce CXC chemokine ligand 8 (CXCL8) when infected with strains of *Salmonella* spp. that are both invasive and flagellated [48]. CXCL8 may serve to attract neutrophils to the site of infection, furthering the inflammatory milieu [16] As a result, the rate of blood-borne DC precursors migrating into the tissues and becoming DCs will increase. These cells will not have been subjected to IEC conditioning and can be directly activated by a combination of pathogens that have reached the epithelial cell barrier and the pro-inflammatory cytokine milieu [16]. In the case of *H. pylori* infection, the initial tolerogenic response is progressively lost, showing that with time the mucosal immune system identified *H. pylori* as a pathogen [49, 50].

3.3 Immune Response to *H. pylori*

H. pylori successfully establishes a chronic infection by achieving a delicate balance between inducing immune responses and surviving in the inflammatory milieu by using an array of important virulence factors. In this part, the mechanisms by which *H. pylori* evades immune-mediated clearance will be discussed in detail, which has been most adopted from Lina et al.'s review article [2]. The next innate and adaptive immune responses to *H. pylori* will be discussed.

3.3.1 Immune Evasion

As discoveries about innate and adaptive immunities are continued, it can be inferred that innate and adaptive immunity via an antigen-specific route are activated once various TLRs are stimulated by microorganism by-products as host-resistance mechanism to eliminate bacterial infection. The immunological reaction that *H. pylori* induces on human stomach mucous membrane includes both innate and adaptive immunities that normal bacteria also perform [51] (Fig. 3.1). However, *H. pylori* can survive on stomach epithelial cells for long term, unlike other bacteria, because it has highly regulated techniques to avoid immunological reactions of its host. Host immunity evasion is the most important factor for the chronic survival of *H. pylori* in human stomach mucous membrane; *H. pylori* regulates innate and adaptive immunities for long-term survival [52] (Fig. 3.2). To maintain infection constantly, *H. pylori* performs six mechanisms to overcome innate immunity. It neutralizes gastric acid (Fig. 3.2a) and has flagella and lipopolysaccharide (LPS) that do not stimulate TLR, which is important for innate


a
Low
pH gradient
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Neutral
VacA
Other virulence factors
DupA
IceA
Oip
Adhesins
BabA/SabA
CagA
LPS
TLR4
TLR2
Mucus layer
MyD88
IRAK1
IRAK4
IL-1β, TNF-α
IL-8
IL-1β, IL-8
d
miR-200b
miR-200c
miRNA (miR-155/miR-146a)
IL-1β, IL-8 TNF-α
Nuclear NF-κB
TRAF5, TRAF2, RIP
TAK1, IKKβ, IL-1, IL-8
TNF-α, iNOS
c
IL-1β, TNF-α, IFN-γ
COX-2
PGE2
ROS/RNS
Dcs
IL-8, IL-10
Monocyte
M1
Proinflammatory
Cell damage
Cell proliferation
Macrophage
M2
Anti-inflammatory
Cancer
b
Neutrophil
Monocyte
Blood vessel

Fig. 3.1 Pathogenesis of *Helicobacter pylori* infection and host immune response. (**a**) Bacterial urease neutralizes the gastric pH, enabling the colonization of gastric epithelial cells by the bacteria and their motility in the mucus layer. Adhesion of the bacteria to the gastric epithelium is mediated by BabA and SabA adhesins, allowing the release of factors CagA and VacA into the host cells, which causes a strong systemic immune response and inflammation of the gastric mucosa. *H. pylori* LPS is recognized by Toll-like receptors, mainly TLR4 and TLR2, in cooperation with the adapter molecule MyD88 associated with IRAK1 and IRAK4 that leads to activation of transcription factor NF-κB, activating inflammatory signaling pathways. (**b**) The immune response is also activated, with the recruitment of inflammatory cells at the infection site, inducing the production of various pro- and anti-inflammatory mediators. (**c**) After NF-κB activation, rapid expression of multiple pro-inflammatory cytokines, chemokines such as the tumor necrosis factor (TNF)-alpha and interleukins, and consequently activation of oncogenic pathways may culminate in cancer. (**d**) The expression of some miRNAs is changed by *H. pylori* infection, and the host immune response is regulated accordingly. *LPS* lipopolysaccharides, *IL* interleukin, *COX-2* cyclooxygenase, *RNS* reactive nitrogen species, *ROS* reactive oxygen species, *IFN* interferon (Adapted from Cadamuro et al. [51])

immunity [52] (Fig. 3.2b). TLR4 detects gram-negative LPS, such as *Escherichia coli* (*E. coli*), but it is immunologically anergic to the LPS of *H. pylori*. Moreover, flagellin of mucous bacteria, such as *Salmonella* or *E. coli*, activates TLR5, while *H. pylori* flagellin is not secreted and does not induce inflammation [52]. In terms of adaptive immunity transformation by *H. pylori* toxins, cytotoxin-associated gene A (CagA), which is a product of the *cag* pathogenicity island (PAI), restricts B-cell proliferation (Fig. 3.2c), and vacuolating cytotoxin A (VacA) limits T-cell proliferation (Fig. 3.2d), as host immunity mechanism alterations [52]. Moreover, *H. pylori* survives inside the cell by changing the host immunity mechanism (Fig. 3.2e) and performing genetic rearrangements (Fig. 3.2f) for its constant colonization [52]. In addition, *H. pylori* performs several mechanisms to restrict lymphocyte activity [27] (Fig. 3.3). VacA limits NFAT (nuclear factor of activated T cells) activity to decrease IL-2 production and to restrict T-cell proliferation [27]

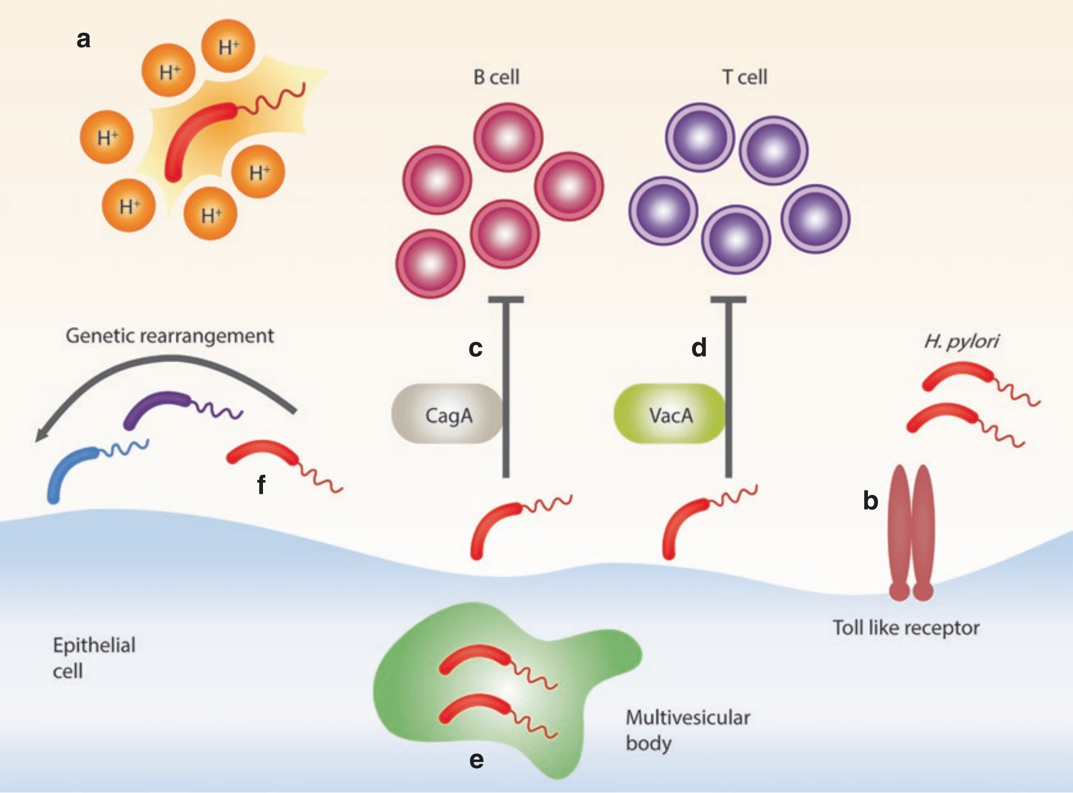

Fig. 3.2 Six mechanisms that *H. pylori* performs to maintain infection. *H. pylori* neutralizes gastric acid to overcome natural immunity (**a**). The bacteria have flagella and lipopolysaccharides that do not stimulate toll-like receptors (**b**). For mutating adaptive immunity, CagA (a product of the cytotoxin-associated gene pathogenicity island (*cag* PAI)) is used to limit B-lymphocyte proliferation (**c**). Also, VacA (vacuolating cytotoxin A) toxin is utilized to limit T-lymphocyte proliferation in terms of adaptive immunity mutation (**d**). In addition, *H. pylori* survives inside cells by altering the host immunity mechanism for sustained colonization (**e**), and the bacteria performs genetic rearrangement (**f**) (Adapted from Merrell and Falkow [52])

(Fig. 3.3). An arginase of *H. pylori* hinders T-cell receptor signal transduction, and low-molecular-weight protein, which is still unclear of its function, restricts T-cell cycle progression to limit T-cell proliferation [27] (Fig. 3.3). These T-cell restrictions lead to the constant *H. pylori* colonization, and MALT lymphoma can occur on some of *H. pylori*-infected patients due to T cell-dependent B-cell proliferation. One of the recent concepts on the evasion mechanism introduces bacterial homologous recombination that responds to host cell oxidative stress due to bacterial invasion. Theoretically, *H. pylori* is an expert on genetic transformation, so the bacteria use homologous recombination to cause antigenic variation for structural transformation of outer membrane protein, such as BabA or BabB, so it evades host cell immunity and induces chronic gastritis [53]. In this review, we discuss how *H. pylori* avoids innate and acquired immune response elements, uses gastric epithelial cells as mediators to manipulate host T-cell responses, and uses virulence factors to avoid adaptive immune responses by T cells to establish a persistent infection as well as the genetic diversity of this pathogen for its survival [1].

3.3.1.1 Inhibition of Innate Immune Recognition by *H. pylori*

Inhibition of innate immune recognition by *H. pylori* includes three aspects: (1) there is avoidance of detection by pattern recognition receptors

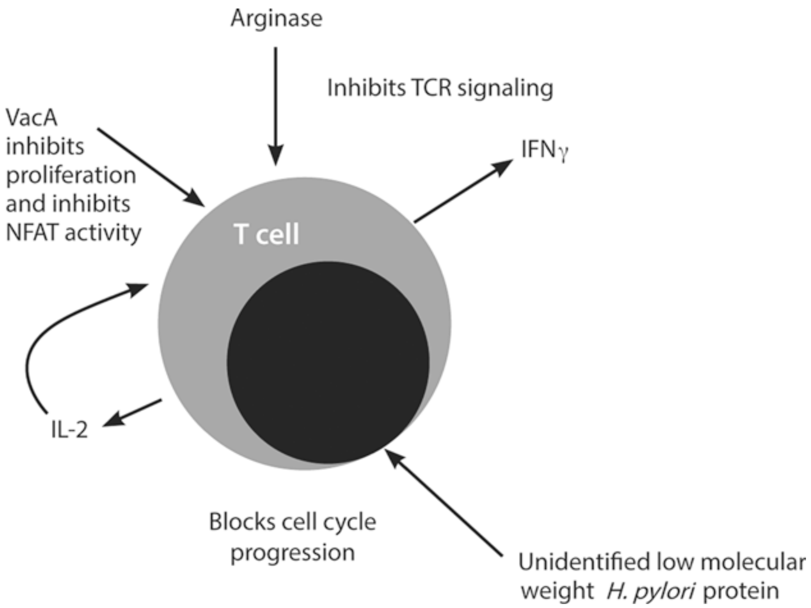
Arginase
Inhibits TCR signaling
VacA inhibits proliferation and inhibits NFAT activity
IFNγ
T cell
IL-2
Blocks cell cycle progression
Unidentified low molecular weight *H. pylori* protein

Fig. 3.3 The mechanism of hindering T-lymphocyte activity by characteristics of *H. pylori*. VacA (vacuolating cytotoxin A) limits NFAT (nuclear factor of activated T cells) activity to decrease IL-2 production and limit T-cell proliferation. Arginase restricts T-cell receptor (*TCR*) signal transduction. Low-molecular-weight protein, which is still unclear of its characteristics, limits the T-cell cycle to restrict T-cell proliferation. *IFN* interferon, *IL* interleukin (Adapted from Algood and Cover [27])

(PRRs); (2) macrophages can engulf *H. pylori,* but the bacterium has developed mechanisms to avoid killing upon phagocytosis [54–56]; and (3) *H. pylori* produces catalase and superoxide dismutase to detoxify ROS [57, 58]. *H. pylori* can also downregulate CXCR1 and CXCR2 expression in human neutrophils, which act as receptors for the neutrophil-recruiting chemokine, IL-8, thus resulting in an inhibitory effect on neutrophil migration and reduced bacterial killing [59].

Evasion of Recognition by Pattern Recognition Receptors

H. pylori evades the innate immune system by a variety of mechanisms. One of these mechanisms is avoidance of detection by PRRs, which are proteins that recognize pathogen-associated molecular patterns (PAMPs) [51]. PAMPs include a large group of molecules that are part of microbes and can vary from microbial surface molecules to nucleic acids. When PRRs recognize PAMPs, they induce several extracellular activation cascades such as the complement pathways and various intracellular signaling pathways, leading to inflammatory responses that are essential for clearance of pathogens [60]. *H. pylori* eludes identification by PRRs by multiple methods, including avoidance of recognition by TLRs and inhibition of c-type lectin (DC-SIGN)-mediated signaling [2]. To avoid recognition by TLRs, the bacterium modulates its surface molecules (including LPS and flagellin). LPS is a glycolipid found on the outer membrane of gram-negative bacteria [61]. It has three distinct units: lipid A, which is responsible for the toxic effects; a core polysaccharide of five sugars linked through ketodeoxyoctulonate to lipid A; and the O-antigen, an outer polysaccharide consisting of up to 25 repeating units of three to five sugars [62]. *H. pylori* expresses O-antigens with great diversity; the bacterium has Lewis antigens, which are made of carbohydrates that resemble human blood group antigens [63]. By exploiting this form of molecular mimicry, the bacterium is able to evade TLRs because the normally detectable O-antigen is recognized as a “self” molecule by this type of PRR. In addition, the bacterium also modifies the lipid A portion of the LPS molecule [2]. Reduced immunogenicity of *H. pylori* LPS could be due to uncommon phosphorylation and acylation of *H. pylori* lipid A [64]. *H. pylori* LPS binds poorly and at a slower rate to LPS-binding proteins, which are acute phase reactants that aid in LPS binding to CD14 and TLR4 on monocytes/macrophages. This reduced binding of LPS to its receptors results in decreased activation of monocyte-macrophages, preventing their contribution to innate immune response. Interestingly, *H. pylori* LPS has also been shown to possess anti-phagocytic properties in vitro [65]. Flagellin is the protein component of bacterial flagella needed for motility and colonization [66]. *H. pylori* relies on five or six polar flagella made of two separate subunits, FlaA and FlaB, to enable movement within the gastric mucus and to counteract peristalsis [67]. TLR5 is a PRR that recognizes flagellin. However, studies showed that *H. pylori* flagellin was not recognized by TLR5 and thus failed to induce NF-κB activation [68]. The study also reported that an eight amino acid stretch in the N-terminal D1 domain of flagellin differed from that of flagellin from bacteria that activated TLR5 [2]. Most flagellated bacteria are able to induce a pro-inflammatory state by promoting the production of IL-8, but *H. pylori* flagellin seems unable to induce IL-8 production in gastric epithelial cells (GECs) [67].

Inhibition of Phagocytic Killing

H. pylori infection activates an inflammatory response in its host, which leads to the recruitment of macrophages, neutrophils, and lymphocytes to the gastric tissue [69]. *H. pylori* can efficiently inhibit its own uptake by these professional phagocytes [1]. This anti-phagocytic phenotype depends on type IV secretion components encoded by the *cag* PAI [70, 71]. Even after macrophages engulf *H. pylori,* the bacterium has developed mechanisms to avoid killing upon phagocytosis [54–56]. Once inside the macrophage, *H. pylori* actively delayed actin polymerization and phagosome formation. *H. pylori*-containing phagosomes then underwent extensive clustering and fusion resulting in the formation of "megasomes" containing multiple bacteria, which caused resistance to intracellular killing [54, 72]. *H. pylori* type I strains were shown to reside in compartments with early endosome properties and did not fuse with lysosomes. VacA alone plays a significant role in the interruption of phagosome maturation [56]. In fact, a study showed that, by interfering with endosomal traffic, VacA altered the presentation of antigens by B cells [73]. A related recent study provided evidence that the effects of VacA on endosomal traffic may prevent the development of a strong Th1 response. The study showed that *H. pylori* VacA could redirect the endocytic pathway of the probiotic bacterium *Lactobacillus acidophilus*, which induces a polarized Th1 response, and does this by blocking the induction of key innate cytokines such as IFN-β and IL-12 [74]. Like other pathogenic bacteria, *H. pylori* also regulates host trafficking pathways by the selective modification of GTPases in macrophages during infection [2]. *H. pylori* has been shown to disrupt the actin cytoskeleton by suppressing *Rgs1/2*, *Fgd2*, and *Dock8*, which are the key regulators of the Rho, Rac, and Cdc42 GTPases, respectively [74]. These are required for the organization and dynamics of actin cytoskeleton needed for proper cell function. This is another mechanism that disrupts phagocyte function and helps *H. pylori* survival in its host [74].

Inhibition of Killing by Reactive Oxygen Species and Nitric Oxide

A major pro-inflammatory factor produced by *H. pylori* is a neutrophil-activating protein (NAP) [75]. *H. pylori* NAP (HP-NAP) is a 150 kDa oligomeric protein, which increases adhesion of polymorphonuclear cells (PMNs) to endothelial cells, stimulates phagocyte chemotaxis, and activates NADPH oxidase to produce reactive oxygen species (ROS) [76, 77]. However, *H. pylori* produces catalase and superoxide dismutase to detoxify ROS [57, 58]. *H. pylori* can also downregulate CXCR1 and CXCR2 expression in human neutrophils, which act as receptors for the neutrophil-recruiting chemokine, IL-8, thus resulting in an inhibitory effect on neutrophil migration and reduced bacterial killing [59]. *H. pylori* also disrupts NADPH oxidase targeting, which was shown to result in the release of superoxide anions in the cytoplasmic membrane instead of the accumulation inside *H. pylori* phagosomes [78]. One antimicrobial host defense mechanism is the generation of nitric oxide (NO) through inducible NO synthase (iNOS). *H. pylori* activates iNOS in macrophages [79]. A mechanism employed by *H. pylori* to activate iNOS involves urease, an important virulence factor of *H. pylori*. Despite the presence of iNOS, *H. pylori* infection persists, which suggests that iNOS production may be at a suboptimal level. *H. pylori* arginase was shown to be an important factor that affords protection of the bacteria against NO-mediated killing since macrophages infected with *H. pylori* produce significantly less NO than arginase isogenic mutants [80]. A recent study showed that induction of macrophage arginase II (Arg2) restricts iNOS protein expression, elicits apoptosis of macrophages as well as pro-inflammatory cytokine production, and limits bacterial killing [81], suggesting another mechanism this bacteria uses to escape macrophage-mediated killing.

3.3.1.2 Modulation of Adaptive Immunity by *H. pylori*

H. pylori have evolved an array of mechanisms to actively dodge adaptive immunity by interfering with antigen presentation and modulation of T-cell responses. Antigen-presenting cells (APCs), represented by macrophages, DCs, and B cells, internalize antigens by phagocytosis or endocytosis and process the antigens and present them to CD4$^+$ T cells via major histocompatibility complex (MHC) class II molecules. This leads to the initiation of antigen-specific T-cell response. The gastric mucosa of *H. pylori*-infected people has an increase in activated macrophages and DCs. Activated macrophages produce IL-6, IL-1β, IL-12, and tumor necrosis factor (TNF)-α, which cause inflammation and help initiate Th1-type responses. In spite of the presence of these effector cells, *H. pylori* successfully establishes a persistent infection, suggesting that these effector cells are unable to clear the pathogen. *H. pylori* has also been shown to cause the polarization of APCs. For instance, during atrophic gastritis macrophages are polarized to the M1 subtype [82]. *H. pylori* can even control the functions of these APCs differently. A study showed that *H. pylori*-mediated activation of DCs and M1 macrophage leads to induction of T-cell proliferation and decreased phagocytosis. On the other hand, upon *H. pylori* infection, the M2 macrophages produced less pro-inflammatory cytokines and increased anti-inflammatory cytokines compared to M1 macrophages [83].

3.3.1.3 Inhibition of Effective T-Cell Response

CD4$^+$ Th cells are major effector cells in the immune response to *H. pylori*. The response was initially characterized as a Th1-polarized response [49, 84], but more recently, other CD4$^+$ T-cell subsets have been found in *H. pylori*-infected patients, and those include Treg and Th17 cells [85–88]. HP-NAP was shown to increase IL-12 and IL-23 production by neutrophils and monocytes, which promote Th1 responses. The addition of HP-NAP to antigen-induced T-cell lines caused a shift from a predominant Th2 to a Th1 phenotype of specific T cells. HP-NAP also elicited an antigen-specific Th1-polarized T-cell response in the gastric mucosa of *H. pylori*-infected patients [89]. Increased production of IFN-γ by Th1 cells was shown to cause chronic gastric inflammation [49, 90]. On the other hand, increased Treg cells produced during *H. pylori* infection suppresses mucosal effector T-cell responses, which contribute to bacterial persistence, and are also a probable cause of gastric tumor progression [85]. Th17 cells, which produce IL-17A, appear to be crucial in the clearance of extracellular bacteria such as *H. pylori* [91]. IL-17 also acts on GEC to release IL-8, a chemokine that recruits neutrophils and thus promotes gastric inflammation. On the other hand, this IL-17-initiated recruitment of neutrophils is critical for the clearance of the bacteria [92]. A hallmark of *H. pylori* infection is that effector T-cell responses are generally impaired during *H. pylori* infection, and T cells from *H. pylori*-infected individuals are hyporesponsive

[93]. As this is an important issue in vaccine design efforts, there has been a significant effort to address mechanisms that impair T-cell responsiveness. *H. pylori* virulence factors that have been reported to play a role in interfering with T-cell responses are VacA, γ-glutamyltranspeptidase (GGT), and arginase [94–99]. Recently, *H. pylori* CagA also has been found to play an important role in modulating Th17 cell response indirectly by modulating expression of B7-H2 on GEC [100].

3.3.1.4 Evasion of Humoral Response

The majority of people infected with *H. pylori* develop a specific antibody response. This response is not normally enough to clear infection. Some studies suggest that infected children produce less antibodies, which may be concurrent with more Treg cells and less activated CD4$^+$ T cells to act as helper cells in the induction of B-cell responses [101]. Although most or all infected individuals are thought to mount an antibody response to *H. pylori*, differences in this response have been noted between those who develop gastritis or duodenal ulcers and those who develop GC [102]. By examining patient serum antibody levels, infected individuals who developed gastritis or duodenal ulcers were shown to have a greater IgG response than those who developed GCs. In turn, GC patients mounted a more vigorous IgA response than those with gastritis and duodenal ulcers. In another study of serum antibody responses to *H. pylori* in Japan, the authors suggested that a weak antibody response was linked to a high risk of developing GC by infected individuals [103]. Another study suggested that the development of antibodies specific to virulence factors of *H. pylori* may be linked to GC [104]. In this study, GC patients were more likely to develop antibodies to CagA and heat shock protein B, while no significant differences were found in the levels of VacA-specific antibodies between individuals with GC and other disease manifestations. These studies suggest that differences in humoral responses to infection may be linked to disease in infected individuals, but the mechanisms behind these differing responses remain elusive. Although most people respond to *H. pylori* with a high serum antibody titer, this response is not efficient in reducing bacterial burden as evidenced by studies in mice that lack B cells and by various vaccine studies. In a study of mice lacking B cells, mice were protected against *H. pylori* challenge suggesting that the humoral response is dispensable in protection against *H. pylori*. In addition to the viewpoint that protection against *H. pylori* challenge is independent of the B-cell response, there is also compelling evidence that antibodies elicited against *H. pylori* may be harmful to the host. One group has shown in mice that specific antibody responses to *H. pylori* may actually aid in bacterial colonization and impair other immune responses against *H. pylori* [105]. This study showed that T cells, not B cells, were responsible for gastritis induced by infection and suggested the possible role of antibodies in inhibiting host resistance to infection in showing improved elimination of bacteria in the absence of antibodies in B cell-deficient mice. B cell-deficient mice were able to clear bacteria at 12–16 weeks postinfection, whereas wild-type mice still had a robust infection coupled with gastritis at this time point. Another compelling study showed that *H. pylori* evades antibody-mediated recognition because of a lack of surface binding of host-elicited antibodies [106]. This study consisted of incubating bacteria with sera from patients who had detectable antibody responses to *H. pylori*. There was very little binding of antibodies to the surface of the bacteria, thus indicating another way the host immune response may be evaded. Another intriguing aspect of the humoral response to *H. pylori* is reports of autoantibodies that are induced during infection. These antibodies were against self-epitopes and potentially caused damage in the host. For instance, *H. pylori* induced antibodies against parietal cells in the stomach, which persisted after bacterial eradication and were linked to intestinal metaplasia [107]. In support of these results, another study examined autoantibodies in infected patient sera, revealing a prevalence of autoantibodies during gastritis associated with gland destruction and gastric atrophy [108]. Decreased acid secretion, but increased gastrin, was seen along with increased gastritis. This was shown in 20% of duodenal ulcer patients coupled with a more severe disease manifestation. Likewise, detrimental effects of autoantibodies have been seen in GC as well. In a small panel of GC patients, spleen cells were isolated and immortalized with human hybridoma technologies, which allowed for the characterization of 11 *H. pylori*-induced autoantibodies that reacted with GC cell-specific proteins [109]. Several of these antibodies stimulated GC cells to proliferate, interestingly enough, in contrast to normal epithelial cells.

3.3.1.5 Genetic Diversity in Immune Evasion

H. pylori is known as one of the most genetically diverse bacterial species since the strain of *H. pylori* 26695 was first sequenced [110]. When 26695 and J99 *H. pylori* strains were compared at the genome level, it was observed that 6% of the genome represented strain-specific genes, which are mostly located in a region now referred to as the plasticity zone [111]. Since then, multiple other strains have supported the observation that such diversity occurs at the size of the genome, gene arrangement, and alleles [2]. This genetic diversity of *H. pylori* was found to be the result of high mutation rates and high recombination frequency [112]. The most well-known genetic diversity is found in two virulent factors, *cagA* and *vacA*. *cagA* is encoded in a 37 kb segment of DNA referred to as the *cag* PAI. An array of *H. pylori* isolates have been noted to differ in the rate with which they have the *cag* PAI in their genome [113], which was recently supported by a study that included 877 isolated from diverse populations and which highlighted the variability in the carriage of *cag* PAI by *H. pylori* strains. This *cag* PAI mostly encodes an array of structural constituents of a bacterial type IV secretion system (T4SS) in addition to a 128 kDa effector protein, CagA. When *H. pylori* adheres to GECs, CagA is translocated via the T4SS into the host cell cytoplasm where it becomes phosphorylated by host cell kinases and interacts with various signaling proteins [114]. As a result of the multiple interactions of CagA with host cell signaling proteins, multiple processes are affected leading to cell transformation [2]. This effector protein, CagA, also has a significant level of diversity, particularly in the C-terminal Glu-Pro-Ile-Tyr-Ala (EPIYA) repeat motifs where CagA is phosphorylated once it is inside the host cell. These EPIYA motifs differ between Asian and Western isolates. An interesting study of *H. pylori* isolates from experimentally infected mice, and nonhuman primates showed that they have rearrangements in CagY of the T4SS [115], which, in turn, results in gain or loss of function in the *H. pylori* T4SS. These observations may be reflective of the overall variability in *H. pylori* strains, which, in turn, contribute to immune escape and the establishment of chronic infection. Another famous toxin in *H. pylori* is VacA, and it shows also frequent genetic polymorphisms. *vacA* encodes a preprotoxin of 139 kDa, which includes an amino-terminal signal peptide and a 50 kDa carboxy-terminal domain that are both cleaved upon secretion to yield a mature toxin monomer of 87–95 kDa [116]. Sequence polymorphisms occur throughout *vacA*, but the two most diverse regions are the signal (s) region, encoding part of the signal peptide, and the N-terminus of the mature protein (which may be type s1 or s2), and the mid (m) region, encoding part of the p58 domain (type m1 or m2) [116]. A third polymorphic determinant of vacuolating activity, the intermediate (i) region, was found to be located between the s-region and m-region within the p37 domain [116]. It has been suggested to be an important determinant of *H. pylori* toxicity and the best independent marker of VacA-associated pathogenicity [116]. As mentioned earlier in this chapter, VacA plays a significant role in the interruption of phagosome maturation [56] and alters the presentation of antigens by B cells by interfering with endosomal traffic [73]. In addition, the effects of VacA on endosomal traffic may prevent the development of a strong Th1 response [2]. From this background, *vacA* genetic diversity could play a role in the immune evasion of *H. pylori* although there is not enough evidence so far.

3.3.2 Innate Immunity Activation Due to *H. pylori*

H. pylori enters the gastric mucosa and reaches the gastric epithelium surface that has a pH of 5 or 6. At this time, the human body activates innate immunity. Various factors like TLR and NodI derive innate immunity reactions to respond to LPS, flagellin, and peptidoglycan of bacteria [51] (Fig. 3.1), and antibacterial protein is secreted from gastric epithelial cells. In detail, peptidoglycan fragments that are delivered by *H. pylori cag* PAI reach the gastric epithelium and are detected by NodI inside the cytoplasm, and this detection acts as an important role when epithelial cells get rid of *H. pylori* directly [117]. Gastric epithelial cells produce various antibacterial proteins, such as α, β defensins, and cathelicidin LL-37 [118, 119], which restrict bacterial growth and take important parts in the host defense mechanism [120, 121]. At the same time, *H. pylori* derives inflammation-inducing gene expression of host cells via TLR; TLR4 detects LPS of bacteria and TLR2 detects peptidoglycan [122]. However, *H. pylori* flagellin is not detected by TLR5 of a host cell, unlike gram-negative *Salmonella enterica*, so the flagellin contributes to the immunity evasion of *H. pylori* [123].

As a result of innate immunity due to *H. pylori* invasion, gastric epithelial cells secrete cytokines that accelerate gastritis occurrence and various immune cell influxes, such as neutrophil [51] (Fig. 3.1). Neutrophil entrance can be observed at the beginning of bacterial influx on the stomach. However, the entrance can also be well observed among chronic *H. pylori* infection during adulthood because cytokines that are derived by *H. pylori*, such as IL-8 and growth-related oncogene (GRO)-α, activate neutrophils and regulate their movement [59]. VacA and HP-NAP of bacteria activate mast cells and increase inflammation [27]. HP-NAP works on TLR2 to derive IL-12 and IL-23 expression on neutrophils and monocytes to accelerate *H. pylori*-selective Th1 immunological reaction. In particular, IL-12 converts a naive Th cell into the Th1 phase [124]. In addition, HP-NAP delays phagosome formation, which helps *H. pylori* from being eliminated by macrophages, so *H. pylori* proliferates inside epithelial and DCs [125]. Moreover, IL-10 that is produced from gastric epithelial cells has the ability to restrict Th1 immunological reaction, so it aids *H. pylori* in avoiding the host immunity as well [27].

3.3.3 Adaptive Immunity Activation Due to *H. pylori*

H. pylori makes DC, T cell, and B cell interact to activate adaptive immunity. Both humoral and cellular immunological reactions are induced and cause systemic or local immunological reactions to produce IgA, IgM, and IgG. Specifically, serum IgM antibody usually appears during the first month of infection [39], serum IgA and IgG antibodies appear as they react with different antigenic determinants of *H. pylori*, and idiosyncratic secretive IgA antibody exists in gastric fluid as a local immunological reaction. Moreover, immunity leads to inflammatory reactions by polymorphonuclear leukocytes and monocytes to secrete various cytokines, like IL-1β, TNF-α, IL-8, and IL-6. Remarkably, Th1 cell is activated once IL-12 is secreted from macrophage and DC, and then cytokines are produced, such as IFN-γ. A macrophage stimulated by *H. pylori* produces a pro-inflammatory substance called nitric oxide by iNOS and NOS2, but *H. pylori* also has *rocF* gene that neutralizes bactericidal action of NO. In other words, the *rocF* gene encodes arginase to use arginine, so it relatively exhausts the iNOS substrate (L-arg) of macrophage [80, 126] (Fig. 3.4). As a result, nitric oxide production by macrophages is relatively reduced, so *H. pylori* can protect itself from host immunity with pro-inflammatory substances.

T cell is the most important factor in immunological reaction to *H. pylori* infection [127]. It is well known that IFN-γ, IL-17, and TNF-α are produced when Th1 and Th7 cells are activated by *H. pylori* infection. However, the recent research has figured out that HP0175 is an important factor for Th17 reaction on mucous membrane [128]. HP0175 increases mRNA of IL-23

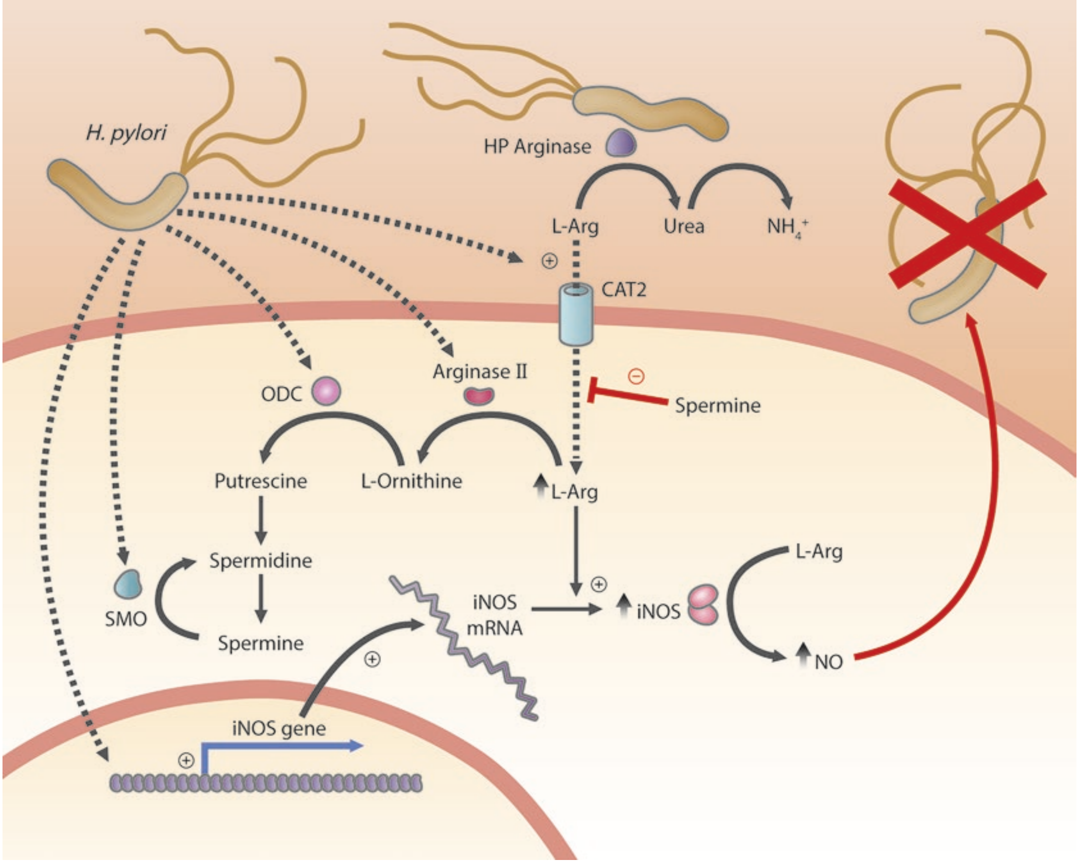

Fig. 3.4 Diagram that explains iNOS production and regulatory reaction on nitric oxide production by macrophage as a result of *H. pylori* stimulation. Once *H. pylori* stimulates a macrophage, arginase II is activated. However, the *H. pylori rocF* gene encodes arginase to utilize arginine for the relative exhaustion of L-arg of iNOS on the macrophage. Then, nitric oxide production by macrophages is relatively reduced. *CAT2* cationic amino acid transporter 2, *ODC* ornithine decarboxylase, *SMO* spermine oxidase (Adapted from Wilson and Crabtree [126])

and IL-12 via TLR4; increases IL-6, IL-1β, and TGF-β production [129]; and accentuates polarized Th17 reaction by IL-17 and IL-21 production increment [130].

Meanwhile, the Th1/Th2 immunological reaction balance is important for etiologies of *H. pylori*-related diseases and the host defense mechanism. If the Th1 immunological reaction is superior, tissue damage is also superior, and *H. pylori* can be eradicated. However, Th2 immunological reaction induces a defense mechanism regarding stomach inflammation, less stomach epithelial cells are damaged, and *H. pylori* can survive, so it leads to chronic infection. Treg cell is important for adaptive immunity regulation [131]; the Treg cell not only regulates and protects pathological changes in the stomach epithelium but also induces chronic infection [132].

3.3.4 Interaction of *H. pylori* with Tight Junction Proteins

Tight junctions (TJs) play a critical role in gastric mucosal defense mechanisms by preserving epithelial monolayer barrier integrity via intracellular adhesions and their interactions in the gastric mucosa, which contains gastric acids, food, and gut microbiota, including *H. pylori* [133]. TJs consist of transmembrane proteins such as occludin (OCLN), junctional adhesion molecules (JAMs), claudins (CLDNs), and cytoplasmic pro-

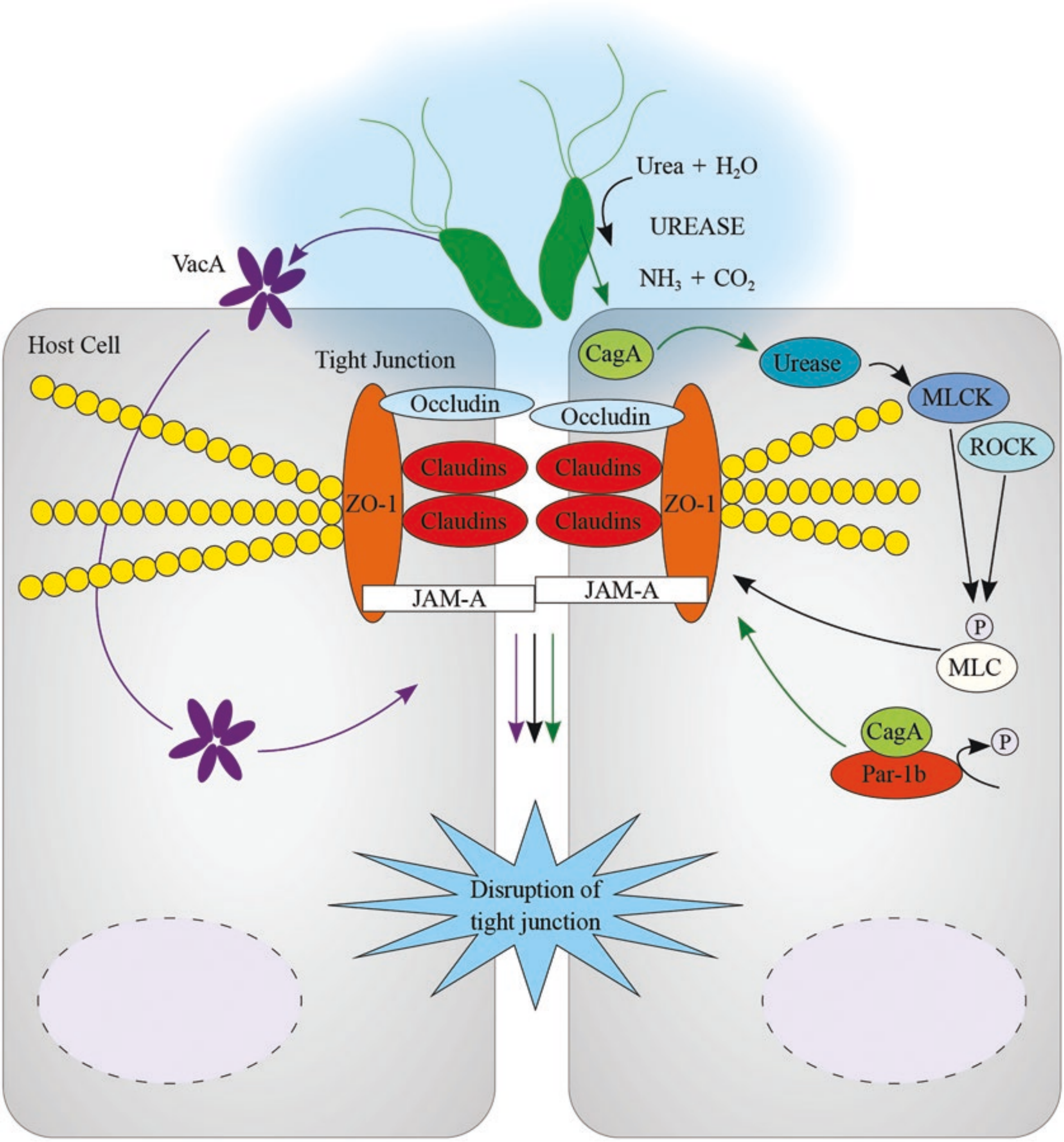
Urea + H_2O
UREASE
NH_3 + CO_2
VacA
Host Cell
Tight Junction
CagA
Urease
MLCK
ROCK
Occludin
Occludin
Claudins
Claudins
Claudins
Claudins
ZO-1
ZO-1
JAM-A
JAM-A
P
MLC
CagA
Par-1b
P
Disruption of tight junction

Fig. 3.5 The cascade events during the invasion of *H. pylori* into tight junctions. *H. pylori* preferentially binds in close proximity to the tight junction and disrupts gastric barrier function, cell adhesion, and cell polarity, which culminates in an invasive phenotype. Tight junctions are composed of the integral membrane proteins occludin, claudins, and junctional adhesion molecule (JAM)-A, as well as zonula occludens-1 (ZO-1). Tight junction function is disrupted by urease activity and phosphorylation of myosin light chain (MLC) by myosin light chain kinase (MLCK) or Rho kinase (ROCK). Translocated CagA interacts with partitioning-defective 1 (PAR1) to inhibit phosphorylation by blocking PAR1 kinase activity and disrupting the tight junction. VacA also increases tight junction permeability (Adapted from Wroblewski and Peek [135])

teins, which bind to transmembrane proteins and act as adaptors and signaling molecules, as is the case of zonula occludens (ZO) [134]. Through cell–cell contacts, the gastric epithelium forms a barrier to protect underlying tissue from pathogenic bacteria; however, *H. pylori* have evolved numerous strategies to perturb the integrity of the gastric barrier [135] (Fig. 3.5). That is, *H. pylori* preferentially bind in close proximity to the tight junction and disrupt gastric barrier function, cell adhesion, and cell polarity, which culminates in an invasive phenotype [135].

Upon injury or death of a cell within the epithelial cell layer, the disruption of TJs in adjacent healthy cells allows growth factors to bind and activate their receptors, thereby inducing cellular proliferation and migration to repair the wound [133]. Dysregulation of TJ proteins (TJPs) leads to the loss of cell-to-cell association, resulting in uncontrolled growth and loss of adhesion between epithelial cells [136], similar to that observed in inflammatory bowel disease [137]. In the case of GC, the expression of TJPs changes to unusual patterns, which affects prognosis or metastasis [133]. For example, CLDN1, 3, and 4 are strongly expressed in intestinal-type adenocarcinoma, and the strong expression of CLDN4 is associated with decreased survival [138, 139]. Moreover, compared to non-neoplastic mucosal tissues, CLDN7 and 8 are upregulated in GC and are related to lymphatic metastasis [140].

The tumor microenvironment (TME) consists of cancer cells and various other components, including infiltrating immune cells, blood vessels, signaling molecules, and extracellular matrix proteins [141]. Such environmental factors induce cancerous status and affect cancer treatment and recurrence [142].

Owing to their key role in regulating cell-to-cell interactions, TJPs are regarded as an important component in TME [143, 144]. Normal tissue adjacent to the tumor is commonly used as a control in cancer studies. Recent studies on the transcriptomic profiling of NAT have revealed that it exists in a unique intermediate state between healthy tissues and tumors [145, 146].

3.4 Conclusion

H. pylori infection is typically acquired during childhood and usually becomes a lifelong infection if left untreated [147]. Colonization by *H. pylori* induces various and complicated natural and adaptive immunity on gastric mucous membranes, creating a chronically inflamed environment with reduced gastric acidity that favors the growth of other bacteria in the gastric environment. However, this host immune response fails to clear the infection because the immune evasion manipulated by *H. pylori* is the most adroit. Nowadays, the interaction between *H. pylori* and non-*H. pylori* became very important in the understanding of *H. pylori*–induced immune mechanism. In addition, the disruption of TJs in adjacent healthy cells allows growth factors to bind and activate their receptors, thereby inducing cellular proliferation and migration to repair the wound. If these responses are inadequate, then the tumor microenvironment is slowly formed. The reason for delayed *H. pylori* vaccine development originated from the complexity of the immunological reactions on *H. pylori* infection and the skillful host immunity evasion mechanisms of the bacteria [148].

References

1. Chen CC, Liou JM, Lee YC, Hong TC, El-Omar EM, Wu MS. The interplay between *Helicobacter pylori* and gastrointestinal microbiota. Gut Microbes. 2021;13:1.
2. Lina TT, Alzahrani S, Gonzalez J, Pinchuk IV, Beswick EJ, Reyes VE. Immune evasion strategies used by *Helicobacter pylori*. World J Gastroenterol. 2014;20:12753–66.
3. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444:1027–31.
4. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell. 2004;118:229–41.
5. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell. 2005;122:107–18.
6. Hattori M, Taylor TD. The human intestinal microbiome: a new frontier of human biology. DNA Res. 2009;16:1–12.
7. Dekio I, Hayashi H, Sakamoto M, Kitahara M, Nishikawa T, Suematsu M, et al. Detection of potentially novel bacterial components of the human skin microbiota using culture-independent molecular profiling. J Med Microbiol. 2005;54(Pt 12):1231–8.
8. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature. 2009;457:480–4.

9. Verma R, Verma AK, Ahuja V, Paul J. Real-time analysis of mucosal flora in patients with inflammatory bowel disease in India. J Clin Microbiol. 2010;48:4279–82.
10. Noor SO, Ridgway K, Scovell L, Kemsley EK, Lund EK, Jamieson C, et al. Ulcerative colitis and irritable bowel patients exhibit distinct abnormalities of the gut microbiota. BMC Gastroenterol. 2010;10:134.
11. Li XX, Wong GL, To KF, Wong VW, Lai LH, Chow DK, et al. Bacterial microbiota profiling in gastritis without *Helicobacter pylori* infection or non-steroidal anti-inflammatory drug use. PLoS One. 2009;4:e7985.
12. Bik EM, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, et al. Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci U S A. 2006;103:732–7.
13. Andersson AF, Lindberg M, Jakobsson H, Bäckhed F, Nyrén P, Engstrand L. Comparative analysis of human gut microbiota by barcoded pyrosequencing. PLoS One. 2008;3:e2836.
14. Sheh A, Fox JG. The role of the gastrointestinal microbiome in *Helicobacter pylori* pathogenesis. Gut Microbes. 2013;4:505–31.
15. Kim J, Kim N, Jo HJ, Park JH, Nam RH, Seok YJ, et al. An appropriate cutoff value for determining the colonization of *Helicobacter pylori* by the pyrosequencing method: comparison with conventional methods. Helicobacter. 2015;20:370–80.
16. Moyat M, Velin D. Immune responses to *Helicobacter pylori* infection. World J Gastroenterol. 2014;20:5583–93.
17. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Introduction of intestinal Th17 cells by segmented filamentous bacteria. Cell. 2009;139:485–98.
18. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, et al. Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. Nature. 2013;500:232–6.
19. Zelante T, Iannitti RG, Cunha C, De Luca A, Giovannini G, Pieraccini G, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity. 2013;39:372–85.
20. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature. 2009;461:1282–6.
21. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A. 2008;105:16731–6.
22. Suzuki K, Meek B, Doi Y, Muramatsu M, Chiba T, Honjo T, et al. Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. Proc Natl Acad Sci U S A. 2004;101:1981–6.
23. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science. 2012;336:1268–73.
24. Donia MS, Fischbach MA. Small molecules from the human microbiota. Science. 2015;349:1254766.
25. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci. 2012;13:701–12.
26. Szczepanik M. Interplay between *Helicobacter pylori* and the immune system. Clinical implications. J Physiol Pharmacol. 2006;57 (Suppl 3):15–27.
27. Algood HM, Cover TL. *Helicobacter pylori* persistence: an overview of interactions between *H. pylori* and host immune defenses. Clin Microbiol Rev. 2006;19:597–613.
28. Cerutti A, Chen K, Chorny A. Immunoglobulin responses at the mucosal interface. Annu Rev Immunol. 2011;29:273–93.
29. Benveniste J, Lespinats G, Salomon J. Serum and secretory IgA in axenic and holoxenic mice. J Immunol. 1971;107:1656–62.
30. Benveniste J, Lespinats G, Adam C, Salomon JC. Immuno-globulins in intact, immunized, and contaminated axenic mice: study of serum IgA. J Immunol. 1971;107:1647–55.
31. Kaneko T, Ota H, Hayama M, Akamatsu T, Katsuyama T. *Helicobacter pylori* infection produces expression of a secretory component in gastric mucous cells. Virchows Arch. 2000;437:514–20.
32. Isaacson P. Immunoperoxidase study of the secretory immunoglobulin system and lysozyme in normal and diseased gastric mucosa. Gut. 1982;23:578–88.
33. Ahlstedt I, Lindholm C, Lönroth H, Hamlet A, Svennerholm AM, Quiding-Järbrink M. Role of local cytokines in increased gastric expression of the secretory component in *Helicobacter pylori* infection. Infect Immun. 1999;67:4921–5.
34. Meyer F, Wilson KT, James SP. Modulation of innate cytokine responses by products of *Helicobacter pylori*. Infect Immun. 2000;68:6265–72.
35. Quiding-Järbrink M, Sundström P, Lundgren A, Hansson M, Bäckström M, Johansson C, et al. Decreased IgA antibody production in the stomach of gastric adenocarcinoma patients. Clin Immunol. 2009;131:463–71.
36. Birkholz S, Schneider T, Knipp U, Stallmach A, Zeitz M. Decreased *Helicobacter pylori*-specific gastric secretory IgA antibodies in infected patients. Digestion. 1998;59:638–45.
37. Macpherson AJ, Gatto D, Sainsbury E, Harriman GR, Hengartner H, Zinkernagel RM. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science. 2000;288:2222–6.
38. Zimmermann K, Haas A, Oxenius A. Systemic antibody responses to gut microbes in health and disease. Gut Microbes. 2012;3:42–7.
39. Nurgalieva ZZ, Conner ME, Opekun AR, Zheng CQ, Elliott SN, Ernst PB, et al. B-cell and T-cell immune

responses to experimental *Helicobacter pylori* infection in humans. Infect Immun. 2005;73:2999–3006.

40. Kao JY, Zhang M, Miller MJ, Mills JC, Wang B, Liu M, et al. *Helicobacter pylori* immune escape is mediated by dendritic cell-induced Treg skewing and Th17 suppression in mice. Gastroenterology. 2010;138:1046–54.
41. Scheinecker C, McHugh R, Shevach EM, Germain RN. Constitutive presentation of a natural tissue autoantigen exclusively by dendritic cells in the draining lymph node. J Exp Med. 2002;196:1079–90.
42. Feng T, Elson CO. Adaptive immunity in the host-microbiota dialog. Mucosal Immunol. 2011;4:15–21.
43. Konrad A, Cong Y, Duck W, Borlaza R, Elson CO. Tight mucosal compartmentation of the murine immune response to antigens of the enteric microbiota. Gastroenterology. 2006;130:2050–9.
44. Coombes JL, Powrie F. Dendritic cells in intestinal immune regulation. Nat Rev Immunol. 2008;8:435–46.
45. Rescigno M, Lopatin U, Chieppa M. Interactions among dendritic cells, macrophages, and epithelial cells in the gut: implications for immune tolerance. Curr Opin Immunol. 2008;20:669–75.
46. Rescigno M, Di Sabatino A. Dendritic cells in intestinal homeostasis and disease. J Clin Invest. 2009;119:2441–50.
47. Rimoldi M, Chieppa M, Salucci V, Avogadri F, Sonzogni A, Sampietro GM, et al. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. Nat Immunol. 2005;6:507–14.
48. Rimoldi M, Chieppa M, Larghi P, Vulcano M, Allavena P, Rescigno M. Monocyte-derived dendritic cells activated by bacteria or by bacteria-stimulated epithelial cells are functionally different. Blood. 2005;106:2818–26.
49. D'Elios MM, Manghetti M, De Carli M, Costa F, Baldari CT, Burroni D, et al. T helper 1 effector cells specific for *Helicobacter pylori* in the gastric antrum of patients with peptic ulcer disease. J Immunol. 1997;158:962–7.
50. Luzza F, Parrello T, Monteleone G, Sebkova L, Romano M, Zarrilli R, et al. Up-regulation of IL-17 is associated with bioactive IL-8 expression in *Helicobacter pylori*-infected human gastric mucosa. J Immunol. 2000;165:5332–7.
51. Cadamuro AC, Rossi AF, Maniezzo NM, Silva AE. *Helicobacter pylori* infection: host immune response, implications on gene expression and microRNAs. World J Gastroenterol. 2014;20:1424–37.
52. Merrell DS, Falkow S. Frontal and stealth attack strategies in microbial pathogenesis. Nature. 2004;430:250–6.
53. Hanada K, Yamaoka Y. Genetic battle between *Helicobacter pylori* and humans. The mechanism underlying homologous recombination in bacteria, which can infect human cells. Microbes Infect. 2014;16:833–9.
54. Allen LA, Schlesinger LS, Kang B. Virulent strains of *Helicobacter pylori* demonstrate delayed phagocytosis and stimulate homotypic phagosome fusion in macrophages. J Exp Med. 2000;191:115–28.
55. Allen LA. Phagocytosis and persistence of *Helicobacter pylori*. Cell Microbiol. 2007;9:817–28.
56. Zheng PY, Jones NL. *Helicobacter pylori* strains expressing the vacuolating cytotoxin interrupt phagosome maturation in macrophages by recruiting and retaining TACO (coronin 1) protein. Cell Microbiol. 2003;5:25–40.
57. Odenbreit S, Wieland B, Haas R. Cloning and genetic characterization of *Helicobacter pylori* catalase and construction of a catalase-deficient mutant strain. J Bacteriol. 1996;178:6960–7.
58. Spiegelhalder C, Gerstenecker B, Kersten A, Schiltz E, Kist M. Purification of *Helicobacter pylori* superoxide dismutase and cloning and sequencing of the gene. Infect Immun. 1993;61:5315–25.
59. Schmausser B, Josenhans C, Endrich S, Suerbaum S, Sitaru C, Andrulis M, et al. Downregulation of CXCR1 and CXCR2 expression on human neutrophils by *Helicobacter pylori*: a new pathomechanism in *H. pylori* infection? Infect Immun. 2004;72:6773–9.
60. Lee MS, Kim YJ. Signaling pathways downstream of pattern-recognition receptors and their cross talk. Annu Rev Biochem. 2007;76:447–80.
61. Cullen TW, Giles DK, Wolf LN, Ecobichon C, Boneca IG, Trent MS. *Helicobacter pylori* versus the host: remodeling of the bacterial outer membrane is required for survival in the gastric mucosa. PLoS Pathog. 2011;7:e1002454.
62. Levinson W. Review of medical microbiology and immunology. 12th ed. New York: McGraw-Hill; 2012.
63. Wang G, Ge Z, Rasko DA, Taylor DE. Lewis antigens in *Helicobacter pylori*: biosynthesis and phase variation. Mol Microbiol. 2000;36:1187–96.
64. Moran AP. Molecular structure, biosynthesis, and pathogenic roles of lipopolysaccharides. In: Mobley HLT, Mendz G, Hazell SL, editors. *Helicobacter pylori*: physiology and genetics. Washington, DC: ASM Press; 2001. p. 81–96.
65. Grebowska A, Moran AP, Matusiak A, Bak-Romaniszyn L, Czkwianianc E, Rechciński T, et al. Anti-phagocytic activity of *Helicobacter pylori* lipopolysaccharide (LPS)—possible modulation of the innate immune response to these bacteria. Pol J Microbiol. 2008;57:185–92.
66. Brooks GF, Butel JS, Morse SA, Mietzner TA. Cell structure. In: Brooks KCCGF, Butel JS, Morse SA, Mietzner TA, editors. Jawetz, Melnick, Adelberg's medical microbiology. 26th ed. New York: McGraw-Hill; 2013. p. 8–41.
67. Gewirtz AT, Yu Y, Krishna US, Israel DA, Lyons SL, Peek RM Jr. *Helicobacter pylori* flagellin evades toll-like receptor 5-mediated innate immunity. J Infect Dis. 2004;189:1914–20.

68. Andersen-Nissen E, Smith KD, Strobe KL, Barrett SL, Cookson BT, Logan SM, et al. Evasion of toll-like receptor 5 by flagellated bacteria. Proc Natl Acad Sci U S A. 2005;102:9247–52.
69. Telford JL, Covacci A, Rappuoli R, Chiara P. Immunobiology of *Helicobacter pylori* infection. Curr Opin Immunol. 1997;9:498–503.
70. Ramarao N, Gray-Owen SD, Backert S, Meyer TF. *Helicobacter pylori* inhibits phagocytosis by professional phagocytes involving type IV secretion components. Mol Microbiol. 2000;37:1389–404.
71. Ramarao N, Meyer TF. *Helicobacter pylori* resists phagocytosis by macrophages: quantitative assessment by confocal microscopy and fluorescence-activated cell sorting. Infect Immun. 2001;69:2604–11.
72. Rittig MG, Shaw B, Letley DP, Thomas RJ, Argent RH, Atherton JC. *Helicobacter pylori*-induced homotypic phagosome fusion in human monocytes is independent of the bacterial vacA and cag status. Cell Microbiol. 2003;5:887–99.
73. Molinari M, Salio M, Galli C, Norais N, Rappuoli R, Lanzavecchia A, et al. Selective inhibition of Ii-dependent antigen presentation by *Helicobacter pylori* toxin VacA. J Exp Med. 1998;187:135–40.
74. Weiss G, Forster S, Irving A, Tate M, Ferrero RL, Hertzog P, et al. *Helicobacter pylori* VacA suppresses lactobacillus acidophilus-induced interferon beta signaling in macrophages via alterations in the endocytic pathway. mBio. 2013;4:e00609–12.
75. Evans DJ Jr, Evans DG, Takemura T, Nakano H, Lampert HC, Graham DY, et al. Characterization of a *Helicobacter pylori* neutrophil-activating protein. Infect Immun. 1995;63:2213–20.
76. Polenghi A, Bossi F, Fischetti F, Durigutto P, Cabrelle A, Tamassia N, et al. The neutrophil-activating protein of *Helicobacter pylori* crosses endothelia to promote neutrophil adhesion in vivo. J Immunol. 2007;178:1312–20.
77. Satin B, Del Giudice G, Della Bianca V, Dusi S, Laudanna C, Tonello F, et al. The neutrophil-activating protein (HP-NAP) of *Helicobacter pylori* is a protective antigen and a major virulence factor. J Exp Med. 2000;191:1467–76.
78. Allen LA, Beecher BR, Lynch JT, Rohner OV, Wittine LM. *Helicobacter pylori* disrupts NADPH oxidase targeting in human neutrophils to induce extracellular superoxide release. J Immunol. 2005;174:3658–67.
79. Lewis ND, Asim M, Barry DP, Singh K, de Sablet T, Boucher JL, et al. Arginase II restricts host defense to *Helicobacter pylori* by attenuating inducible nitric oxide synthase translation in macrophages. J Immunol. 2010;184:2572–82.
80. Gobert AP, McGee DJ, Akhtar M, Mendz GL, Newton JC, Cheng Y, et al. *Helicobacter pylori* arginase inhibits nitric oxide production by eukaryotic cells: a strategy for bacterial survival. Proc Natl Acad Sci U S A. 2001;98:13844–9.
81. Lewis ND, Asim M, Barry DP, de Sablet T, Singh K, Piazuelo MB, et al. Immune evasion by *Helicobacter pylori* is mediated by induction of macrophage arginase II. J Immunol. 2011;186:3632–41.
82. Quiding-Järbrink M, Raghavan S, Sundquist M. Enhanced M1 macrophage polarization in human *Helicobacter pylori*-associated atrophic gastritis and in vaccinated mice. PLoS One. 2010;5:e15018.
83. Fehlings M, Drobbe L, Moos V, Renner Viveros P, Hagen J, Beigier-Bompadre M, et al. Comparative analysis of the interaction of *Helicobacter pylori* with human dendritic cells, macrophages, and monocytes. Infect Immun. 2012;80:2724–34.
84. Bamford KB, Fan X, Crowe SE, Leary JF, Gourley WK, Luthra GK, et al. Lymphocytes in the human gastric mucosa during *Helicobacter pylori* have a T helper cell 1 phenotype. Gastroenterology. 1998;114:482–92.
85. Enarsson K, Lundgren A, Kindlund B, Hermansson M, Roncador G, Banham AH, et al. Function and recruitment of mucosal regulatory T cells in human chronic *Helicobacter pylori* infection and gastric adenocarcinoma. Clin Immunol. 2006;121:358–68.
86. Lundgren A, Strömberg E, Sjöling A, Lindholm C, Enarsson K, Edebo A, et al. Mucosal FOXP3-expressing CD^{4+} CD25 high regulatory T cells in *Helicobacter pylori*-infected patients. Infect Immun. 2005;73:523–31.
87. Shi Y, Liu XF, Zhuang Y, Zhang JY, Liu T, Yin Z, et al. *Helicobacter pylori*-induced Th17 responses modulate Th1 cell responses, benefit bacterial growth, and contribute to pathology in mice. J Immunol. 2010;184:5121–9.
88. Serelli-Lee V, Ling KL, Ho C, Yeong LH, Lim GK, Ho B, et al. Persistent *Helicobacter pylori* specific Th17 responses in patients with past *H. pylori* infection are associated with elevated gastric mucosal IL-1β. PLoS One. 2012;7:e39199.
89. Amedei A, Cappon A, Codolo G, Cabrelle A, Polenghi A, Benagiano M, et al. The neutrophil-activating protein of *Helicobacter pylori* promotes Th1 immune responses. J Clin Invest. 2006;116:1092–101.
90. Karttunen R, Karttunen T, Ekre HP, MacDonald TT. Interferon gamma and interleukin 4 secreting cells in the gastric antrum in *Helicobacter pylori* positive and negative gastritis. Gut. 1995;36:341–5.
91. Kabir S. The role of interleukin-17 in the *Helicobacter pylori* induced infection and immunity. Helicobacter. 2011;16:1–8.
92. DeLyria ES, Redline RW, Blanchard TG. Vaccination of mice against *H. pylori* induces a strong Th-17 response and immunity that is neutrophil dependent. Gastroenterology. 2009;136:247–56.
93. Fan XJ, Chua A, Shahi CN, McDevitt J, Keeling PW, Kelleher D. Gastric T lymphocyte responses to *Helicobacter pylori* in patients with *H. pylori* colonisation. Gut. 1994;35:1379–84.
94. Boncristiano M, Paccani SR, Barone S, Ulivieri C, Patrussi L, Ilver D, et al. The *Helicobacter*

pylori vacuolating toxin inhibits T cell activation by two independent mechanisms. J Exp Med. 2003;198:1887–97.

95. Ganten TM, Aravena E, Sykora J, Koschny R, Mohr J, Rudi J, et al. *Helicobacter pylori*-induced apoptosis in T cells is mediated by the mitochondrial pathway independent of death receptors. Eur J Clin Invest. 2007;37:117–25.
96. Schmees C, Prinz C, Treptau T, Rad R, Hengst L, Voland P, et al. Inhibition of T-cell proliferation by *Helicobacter pylori* gamma-glutamyl transpeptidase. Gastroenterology. 2007;132:1820–33.
97. Sundrud MS, Torres VJ, Unutmaz D, Cover TL. Inhibition of primary human T cell proliferation by *Helicobacter pylori* vacuolating toxin (VacA) is independent of VacA effects on IL-2 secretion. Proc Natl Acad Sci U S A. 2004;101:7727–32.
98. Torres VJ, VanCompernolle SE, Sundrud MS, Unutmaz D, Cover TL. *Helicobacter pylori* vacuolating cytotoxin inhibits activation-induced proliferation of human T and B lymphocyte subsets. J Immunol. 2007;179:5433–40.
99. Wang J, Brooks EG, Bamford KB, Denning TL, Pappo J, Ernst PB. Negative selection of T cells by *Helicobacter pylori* as a model for bacterial strain selection by immune evasion. J Immunol. 2001;167:926–34.
100. Lina TT, Pinchuk IV, House J, Yamaoka Y, Graham DY, Beswick EJ, et al. CagA-dependent downregulation of B7-H2 expression on gastric mucosa and inhibition of Th17 responses during *Helicobacter pylori* infection. J Immunol. 2013;191:3838–46.
101. Soares TF, Rocha GA, Rocha AM, Corrêa-Oliveira R, Martins-Filho OA, Carvalho AS, et al. Phenotypic study of peripheral blood lymphocytes and humoral immune response in *Helicobacter pylori* infection according to age. Scand J Immunol. 2005;62:63–70.
102. Manojlovic N, Nikolic L, Pilcevic D, Josifovski J, Babic D. Systemic humoral anti-*Helicobacter pylori* immune response in patients with gastric malignancies and benign gastroduodenal disease. Hepatogastroenterology. 2004;51:282–4.
103. Yamaji Y, Mitsushima T, Ikuma H, Okamoto M, Yoshida H, Kawabe T, et al. Weak response of *Helicobacter pylori* antibody is high risk for gastric cancer: a cross-sectional study of 10,234 endoscoped Japanese. Scand J Gastroenterol. 2002;37:148–53.
104. Iaquinto G, Todisco A, Giardullo N, D'Onofrio V, Pasquale L, De Luca A, et al. Antibody response to *Helicobacter pylori* CagA and heat-shock proteins in determining the risk of gastric cancer development. Dig Liver Dis. 2000;32:378–83.
105. Akhiani AA, Schön K, Franzén LE, Pappo J, Lycke N. *Helicobacter pylori*-specific antibodies impair the development of gastritis, facilitate bacterial colonization, and counteract resistance against infection. J Immunol. 2004;172:5024–33.
106. Darwin PE, Sztein MB, Zheng QX, James SP, Fantry GT. Immune evasion by *Helicobacter pylori*: gastric spiral bacteria lack surface immunoglobulin deposition and reactivity with homologous antibodies. Helicobacter. 1996;1:20–7.
107. Basso D, Gallo N, Zambon CF, Baron M, Navaglia F, Stockreiter E, et al. Antigastric autoantibodies in *Helicobacter pylori* infection: role in gastric mucosal inflammation. Int J Clin Lab Res. 2000;30:173–8.
108. Faller G, Ruff S, Reiche N, Hochberger J, Hahn EG, Kirchner T. Mucosal production of antigastric autoantibodies in *Helicobacter pylori* gastritis. Helicobacter. 2000;5:129–34.
109. Hensel F, Knörr C, Hermann R, Krenn V, Müller-Hermelink HK, Vollmers HP. Mitogenic autoantibodies in *Helicobacter pylori*-associated stomach cancerogenesis. Int J Cancer. 1999;81:229–35.
110. Tomb JF, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, et al. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature. 1997;388:539–47.
111. Alm RA, Ling LS, Moir DT, King BL, Brown ED, Doig PC, et al. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature. 1999;397:176–80.
112. Björkholm B, Sjölund M, Falk PG, Berg OG, Engstrand L, Andersson DI. Mutation frequency and biological cost of antibiotic resistance in *Helicobacter pylori*. Proc Natl Acad Sci U S A. 2001;98:14607–12.
113. Gressmann H, Linz B, Ghai R, Pleissner KP, Schlapbach R, Yamaoka Y, et al. Gain and loss of multiple genes during the evolution of *Helicobacter pylori*. PLoS Genet. 2005;1:e43.
114. Naito M, Yamazaki T, Tsutsumi R, Higashi H, Onoe K, Yamazaki S, et al. Influence of EPIYA-repeat polymorphism on the phosphorylation-dependent biological activity of *Helicobacter pylori* CagA. Gastroenterology. 2006;130:1181–90.
115. Barrozo RM, Cooke CL, Hansen LM, Lam AM, Gaddy JA, Johnson EM, et al. Functional plasticity in the type IV secretion system of *Helicobacter pylori*. PLoS Pathog. 2013;9:e1003189.
116. Rhead JL, Letley DP, Mohammad M, Hussein N, Mohagheghi MA, Hosseini ME, et al. A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. Gastroenterology. 2007;133:926–36.
117. Grubman A, Kaparakis M, Viala J, Allison C, Badea L, Karrar A, et al. The innate immune molecule, NOD1, regulates direct killing of *Helicobacter pylori* by antimicrobial peptides. Cell Microbiol. 2010;12:626–39.
118. Isomoto H, Mukae H, Ishimoto H, Date Y, Nishi Y, Inoue K, et al. Elevated concentrations of alpha-defensins in gastric juice of patients with *Helicobacter pylori* infection. Am J Gastroenterol. 2004;99:1916–23.
119. Hase K, Murakami M, Iimura M, Cole SP, Horibe Y, Ohtake T, et al. Expression of LL-37 by human gastric epithelial cells as a potential host defense mechanism against *Helicobacter pylori*. Gastroenterology. 2003;125:1613–25.

120. Miehlke S, Reddy R, Osato MS, Ward PP, Conneely OM, Graham DY. Direct activity of recombinant human lactoferrin against *Helicobacter pylori*. J Clin Microbiol. 1996;34:2593–4.
121. Dial EJ, Hall LR, Serna H, Romero JJ, Fox JG, Lichtenberger LM. Antibiotic properties of bovine lactoferrin on *Helicobacter pylori*. Dig Dis Sci. 1998;43:2750–6.
122. Smith MF Jr, Novotny J, Carl VS, Comeau LD. *Helicobacter pylori* and toll-like receptor agonists induce syndecan-4 expression in an NF-kappaB-dependent manner. Glycobiology. 2006;16:221–9.
123. Lee SK, Stack K, Katzowitsch E, Aizawa SI, Suerbaum S, Josenhans C. *Helicobacter pylori* flagellins have very low intrinsic activity to stimulate human gastric epithelial cells via TLR5. Microbes Infect. 2003;5:1345–56.
124. Amedei A, Bergman MP, Appelmelk BJ, Azzurri A, Benagiano M, Tamburini C, et al. Molecular mimicry between *Helicobacter pylori* antigens and H^+, K^+-adenosine triphosphatase in human gastric autoimmunity. J Exp Med. 2003;198:1147–56.
125. Wang YH, Gorvel JP, Chu YT, Wu JJ, Lei HY. *Helicobacter pylori* impairs murine dendritic cell responses to infection. PLoS One. 2010;5:e10844.
126. Wilson KT, Crabtree JE. Immunology of *Helicobacter pylori*: insights into the failure of the immune response and perspectives on vaccine studies. Gastroenterology. 2007;133:288–308.
127. O'Keeffe J, Moran AP. Conventional, regulatory, and unconventional T cells in the immunological response to *Helicobacter pylori*. Helicobacter. 2008;13:1–19.
128. D'Elios MM, Czinn SJ. Immunity, inflammation and vaccines for *Helicobacter pylori*. Helicobacter. 2014;19 Suppl 1:19–26.
129. Amedei A, Munari F, Della Bella C, Niccolai E, Benagiano M, Bencini L, et al. *Helicobacter pylori* HP0175 promotes the production of IL-23, IL-6, IL-1β and TGF-β. Eur J Inflamm. 2013;11:261–8.
130. Amedei A, Munari F, Bella CD, Niccolai E, Benagiano M, Bencini L, et al. *Helicobacter pylori* secreted peptidyl prolyl cis, trans-isomerase drives Th17 inflammation in gastric adenocarcinoma. Intern Emerg Med. 2014;9:303–9.
131. Kandulski A, Malfertheiner P, Wex T. Role of regulatory T-cells in *H. pylori*-induced gastritis and gastric cancer. Anticancer Res. 2010;30:1093–103.
132. D'Elios MM, Andersen LP. Inflammation, immunity, and vaccines for *Helicobacter pylori*. Helicobacter. 2009;14 (Suppl 1):21–8.
133. Choi SI, Kim N, Park JH, Nam RH, Song CH, Lee HS. Effect of *Helicobacter pylori* infection and its eradication on the expression of tight junction proteins in the gastric epithelium in relation to gastric carcinogenesis. Helicobacter. 2022;27:e12929.
134. Caron TJ, Scott KE, Fox JG, Hagen SJ. Tight junction disruption: *Helicobacter pylori* and dysregulation of the gastric mucosal barrier. World J Gastroenterol. 2015;21:11411–27.
135. Wroblewski LE, Peek RM. Targeted disruption of the epithelial-barrier by *Helicobacter pylori*. Cell Commun Signal. 2011;9:29.
136. Oh S, Kim N, Kwon JW, Shin CM, Choi YJ, Lee DH, et al. Effect of *Helicobacter pylori* eradication and ABO genotype on gastric cancer development. Helicobacter. 2016;21:596–605.
137. Kuo WT, Zuo L, Odenwald MA, Madha S, Singh G, Gurniak CB, et al. The tight junction protein ZO-1 is dispensable for barrier function but critical for effective mucosal repair. Gastroenterology. 2021;161:1924–39.
138. Resnick MB, Gavilanez M, Newton E, Konkin T, Bhattacharya B, Britt DE, et al. Claudin expression in gastric adenocarcinomas: a tissue microarray study with prognostic correlation. Hum Pathol. 2005;36:886–92.
139. Shareef MM, Radi DM, Eid AM. Tight junction protein claudin 4 in gastric carcinoma and its relation to lymphangiogenic activity. Arab J Gastroenterol. 2015;16:105–12.
140. Yang L, Sun X, Meng X. Differences in the expression profiles of claudin proteins in human gastric carcinoma compared with non neoplastic mucosa. Mol Med Rep. 2018;18:1271–8.
141. Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. J Cell Sci. 2012;125:5591–6.
142. Quante M, Varga J, Wang TC, Greten FR. The gastrointestinal tumor microenvironment. Gastroenterology. 2013;145:63–78.
143. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013;19:1423–37.
144. Martin TA, Jiang WG. Loss of tight junction barrier function and its role in cancer metastasis. Biochim Biophys Acta. 2009;1788:872–91.
145. Aran D, Camarda R, Odegaard J, Paik H, Oskotsky B, Krings G, et al. Comprehensive analysis of normal adjacent to tumor transcriptomes. Nat Commun. 2017;8:1077.
146. Russi S, Calice G, Ruggieri V, Laurino S, La Rocca F, Amendola E, et al. Gastric normal adjacent mucosa versus healthy and cancer tissues: distinctive transcriptomic profiles and biological features. Cancers (Basel). 2019;11:1248.
147. Chong J, Marshall BJ, Barkin JS, McCallum RW, Reiner DK, Hoffman SR, et al. Occupational exposure to *Helicobacter pylori* for the endoscopy professional: a sera epidemiological study. Am J Gastroenterol. 1994;89:1987–92.
148. Zeng M, Mao XH, Li JX, Tong WD, Wang B, Zhang YJ, et al. Efficacy, safety, and immunogenicity of an oral recombinant *Helicobacter pylori* vaccine in children in China: a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2015;386:1457–64.

4

Change of Acid Secretions, Ghrelin, and Leptin, by *H. pylori*

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Abstract

When *Helicobacter pylori* (*H. pylori*) begins to colonize on the surface of the gastric epithelium, *H. pylori* induces strong inflammatory responses and causes transitory hypochlorhydria. The main underlying mechanisms are as follows: first, *H. pylori* represses the activity of promoter of the alpha-subunit (HKα) of H^+, K^+-ATPase, and second, cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α suppress gastric acid. Third, *H. pylori*-induced gastritis evokes the decrease in parietal cell numbers. In the chronic *H. pylori* gastritis, the change of gastric acid is mainly determined by the pattern of corpus gastritis. That is, in the antral-predominant gastritis, acid secretion increases due to an increase in gastrin secretion. In the situation of corpus-predominant gastritis, acid secretion is decreased similar to acute gastritis. The acute response after *H. pylori* eradication includes the increase of gastric acid mainly due to the clearance of *H. pylori* itself and its secreted proteins, reversed activity of H^+, K^+-ATPase, and decrease of IL-1β and TNF-α. After this acute response phenomenon, the change in acid secretion depends on the gastritis pattern. That is, in the situation of corpus-predominant gastritis, acid secretion increases slowly until 2 years depending on the severity of atrophic gastritis. In the antral-predominant gastritis, the increase of gastric acid secretion stops and it returns to normal as gastrin secretion decreases. *H. pylori* infected subjects have lower basal and fasting serum levels of ghrelin and higher concentrations of leptin compared to controls. Since leptin is an anorexigenic hormone, and ghrelin stimulates the release of growth hormone in humans, consequently, *H. pylori* infection reduces appetite. In contrast to acid secretion, the effect of *H. pylori* and its eradication on ghrelin and leptin levels are still controversial.

Keywords

Helicobacter pylori · Acid secretion · Ghrelin
Leptin

4.1 Introduction

One of the important functions of gastric physiology is to regulate and sustain acid secretion to sterilize ingested nutrients in which system sev-

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eral hormones are involved. In regard to gastric acid secretion, gastrin and somatostatin have been well investigated. In the acute phase, *Helicobacter pylori* (*H. pylori*) is capable of inhibiting acid secretion directly as well as indirectly by activating intramural calcitonin gene-related peptide (CGRP) sensory neurons coupled to the stimulation of somatostatin and the inhibition of histamine secretion. Its infection of the human gastric mucosa inevitably alters normal gastric physiology. Particularly, activated cytokines deregulate the secretion of gastric hormones including gastrin, somatostatin, ghrelin, and leptin. Chronic *H. pylori*-induced inflammation changes the gastric hormones and gastric acid depending on the pattern of gastritis such as antral- or corpus-dominant gastritis. *H. pylori* eradication causes the reverse process of gastric physiology which is determined by the severity of atrophy and from the pattern of gastritis. In contrast, ghrelin and leptin which are secreted from the stomach play a very important role in controlling appetite and satiety in an autocrine/paracrine manner in the central nervous system (CNS). They are also affected by *H. pylori*-induced inflammation. Interestingly, this bacterium is able to produce different biological effects on hormones controlling both appetite and growth, mostly depending on the time of acquisition of the infection and of eradication [1]. This chapter aims to review the data on gastric secretion and hormones that are produced in the stomach, focusing on the effect of *H. pylori* infection and its eradication.

4.2 Gastric Acid Secretion and H^+, K^+-ATPase with Regard to *H. pylori* Infection

There are three regulatory molecules (acetylcholine, histamine, and gastrin) that stimulate acid secretion and one regulatory molecule (somatostatin) that inhibits acid secretion [2] (Fig. 4.1). Acetylcholine is a neurotransmitter that is released by enteric neurons, while histamine is a paracrine that is released from enterochromaffin-like (ECL) cells. Gastrin is released from the G cells of the antral mucosa and travels through the bloodstream to the corpus where it stimulates ECL cells to secrete histamine which, in turn, stimulates the parietal cells to secrete acid (Fig. 4.1). In contrast, somatostatin, secretin, gastric inhibitory peptide (GIP), and vasoactive inhibitory peptide (VIP) inhibit acid secretion. Gastrin also has a direct effect on parietal cells, which stimulates parietal cells' proliferation. In oxyntic glands of the gastric body and fundus, somatostatin-releasing D cells are anatomically and functionally coupled to parietal and ECL cells [3] (Fig. 4.2). When the pH of the stomach gets too low, somatostatin secretion is stimulated, and it inhibits acid secretion by direct effects on parietal cells and also by inhibiting the release of the positive regulators, histamine, and gastrin. Inversely, endogenous gastrin release stops when the pH of the perfusate drops below 2.5. Gastrin release is suppressed primarily by direct contact of acid with the antrum [4]. Taken together, acid secretion and physiology of G and D cells are closely related to each other, and the effect of *H. pylori* on these cells is closely related to the acid secretory system. Finally, H^+, K^+-ATPase is an acid pump located in the parietal cells and this pump is largely affected by *H. pylori*. This part focuses on the two major acid regulatory hormones, gastrin and somatostatin, and H^+, K^+-ATPase together with the effect of *H. pylori* infection or eradication on the acid secretion.

4.2.1 Gastric Acid Secretion and H^+, K^+-ATPase

Acid secretion depends on the activation of the gastric H^+, K^+-ATPase, termed as the acid or proton pump. This enzyme was found uniquely in gastric parietal cells and in renal collecting ducts [2]. Parietal cells are located at the oxyntic gastric gland of the corpus (Fig. 4.2). Mg^{2+}-dependent, K^+-stimulated, H_3O^+-transporting, and P-type adenosine triphosphatase (H^+, K^+-ATPase, EC3.6.1.36) [5] consists of an α-subunit and β-subunit [6] (Fig. 4.3). It is an electroneutral H^+ for K^+ exchange P_2-type (phosphorylating)

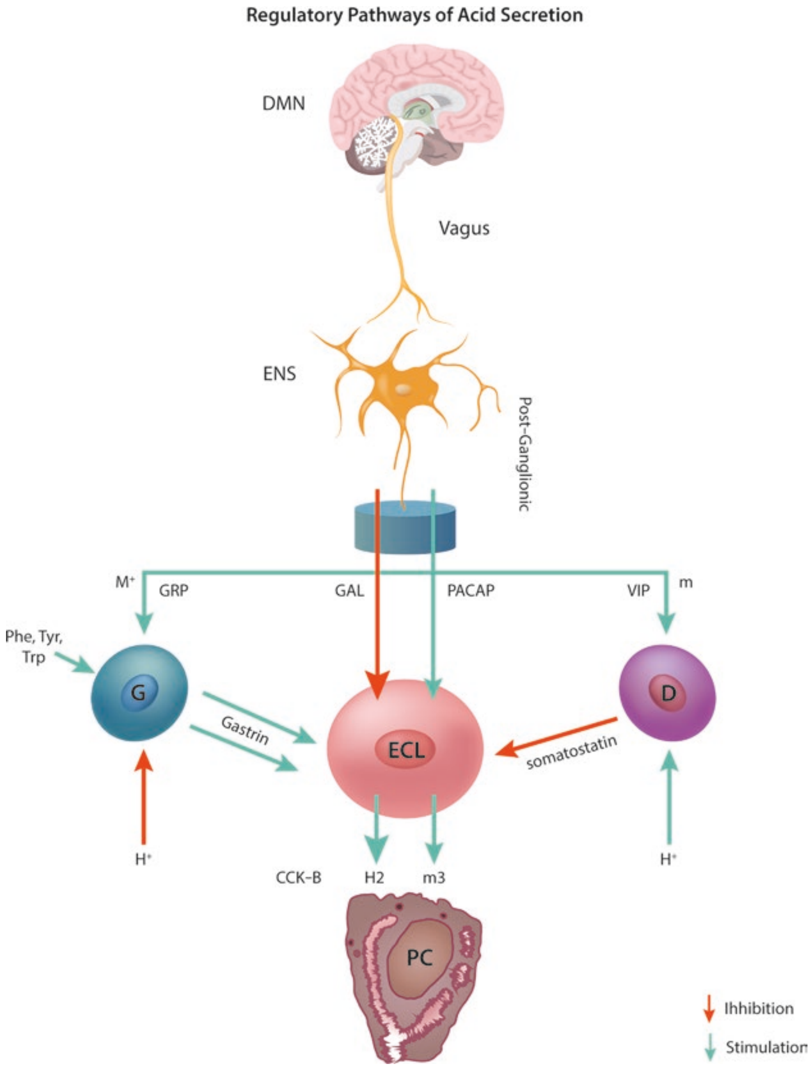

Fig. 4.1 Regulation of gastric acid secretion showing both activating and inhibitory pathways, neural, endocrine, and paracrine. *DMN* dorsal motor nuclei, *ENS* enteric nervous system, *GRP* gastrin-releasing protein, *GAL* gastrin ligand, *PACAP* pituitary adenylate cyclase-activating peptide, *VIP* vasoactive intestinal peptide, *D cell* somatostatin cell, *G cell* gastrin cell, *PC* parietal cell, *H2* histamine receptor type 2, *m3* muscarinic receptor subtype 3 (Adapted from Sachs et al. [2])

ATPase with ten membrane-spanning segments (α-subunit, HKα, Mr. ~94,000) and with one transmembrane segment and six or seven glycosylation sites (β-subunit, HKβ, Mr. 34,000) [6] (Fig. 4.3). The cytoplasmic domain (HKα) with catalytic and transport functions has three loops, the N or nucleotide-binding domain, the A or activation domain, and the P or phosphorylation domain (Fig. 4.3). Human HKα gene resides on chromosome 19 (19q13.1), and HKα contains sequences responsible for apical membrane localization [7]. HKβ gene is located on chromosome 13 (13q34), and HKβ protects the enzyme from degradation and is necessary for trafficking to and from the plasma membrane [8]. The morphology of the parietal cell becomes different depending on the activity of acid secretion [9] (Fig. 4.4). In the resting parietal cell, most of the ATPase is sequestered in cytoplasmic tubulovesicles, as a form of the $\alpha_2\beta_2$ complex, and upon stimulation, it is trafficked to the apical microvilli of the secretory canaliculus [9, 10] during which the surface area increases up to five times [11–13]. Actually parietal cells need a huge amount of energy for the active acid secretion, and as a result, there are high densities of mitochondria, NAD$^+$/NADH, and tubulovesicles in the parietal cells [11–13].

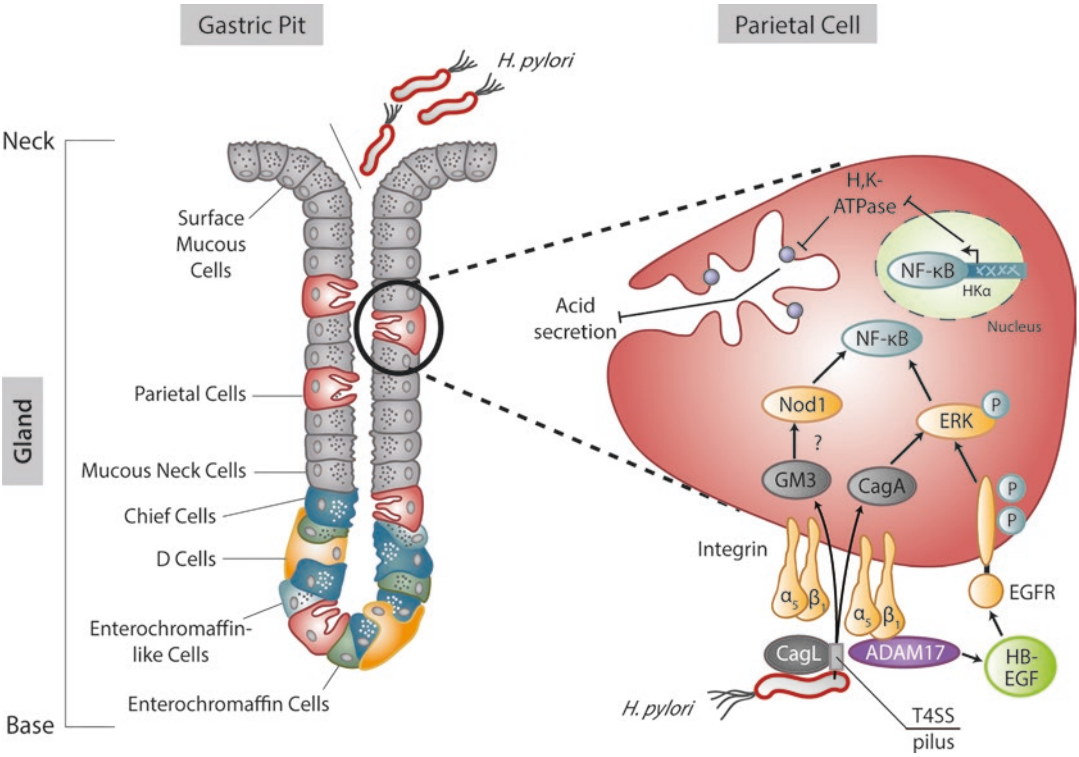
Gastric Pit
Parietal Cell
H. pylori
Neck
Surface Mucous Cells
Gland
Parietal Cells
Mucous Neck Cells
Chief Cells
D Cells
Enterochromaffin-like Cells
Enterochromaffin Cells
Base
H,K-ATPase
NF-κB
HKα
Nucleus
Acid secretion
NF-κB
Nod1
?
ERK
P
GM3
CagA
P
P
Integrin
α5 β1
α5 β1
EGFR
CagL
ADAM17
HB-EGF
H. pylori
T4SS pilus

Fig. 4.2 Schematic illustration of a corporal gland in the human stomach focusing on the acid-secreting region. The location of various cell types in a gland of the human corpus is indicated in different colors. A gastric parietal cell is enlarged to the right, showing *Helicobacter pylori* interacting with integrins through CagL, injecting CagA and possibly the bacterial peptidoglycan-derived glycosylated tripeptide GM-3, leading to the activation of diverse host signaling pathways. The consequent mobilization of nuclear factor (NF)-κB p50 homodimers with the nucleus results in the repression of gastric H^+, K^+-adenosine triphosphatase (H^+, K^+-ATPase) α subunit transcription and the inhibition of acid secretion as indicated. *ADAM 17* a disintegrin and a metalloprotease 17, *EGFR* EGF receptor, *HB-EGF* heparin-binding epidermal growth factor, *T4SS* type IV secretion system (Adapted from Smolka and Backert [3])

4.2.2 Effect of *H. pylori* Infection on the Gastric Acid Secretion

Acute administration of *H. pylori* has been known to be capable of inhibiting acid secretion directly. However, recently there has been a report regarding an indirect pathway by activating intramural CGRP sensory neurons coupled to the stimulation of somatostatin and the inhibition of histamine secretion [14]. Since *H. pylori* is present in the upper regions of the gastric mucosa, whereas ECL cells are located from the base to the neck of glands (Fig. 4.2), it has been speculated that the activation of CGRP sensory neurons may be one of the explanations to how initial patchy superficial colonization of the stomach can induce acute hypochlorhydria [14]. In addition, *H. pylori*-infected patients with acid hyposecretion tend to have corpus gastritis caused by a specific *H. pylori* product or by inflammatory cytokines, including IL-1β and TNF-α, which inhibit parietal cells [15]. IL-1β also inhibits ECL cells [16]. When *H. pylori* infection goes to chronic state, it accelerates the development of corpus atrophy, which further diminishes acid secretion through the loss of parietal cells [17]. A recent study clearly demonstrated that *H. pylori* (*cagA* positive) infection induced a decrease in acid secretion and an increase in serum gastrin, with these phenomena returning to control levels after treatment with an IL-1 receptor antagonist in Mongolian gerbils

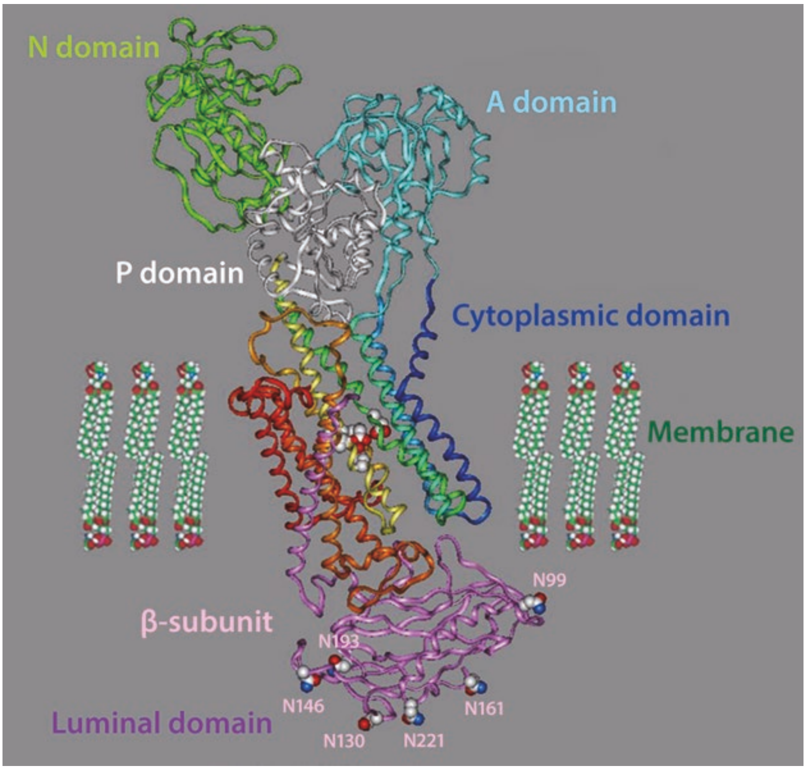

Fig. 4.3 A model structure of the gastric H⁺, K⁺-ATPase. The gastric H⁺, K⁺-ATPase α subunit has three lobes, N (ATP binding), P (phosphorylation), and A (activation) domains in the cytoplasmic domain and three transmembrane segments in the membrane domain. The gastric β subunit has a short cytoplasmic region, one transmembrane segment, and a heavily glycosylated extracellular region. The number of Asn sites having carbohydrates is based on pig H⁺, K⁺-ATPase (Adapted from Shin and Kim [6], with permission from The Korean Society of Neurogastroenterology and Motility)

[18]. Because gastrin is a physiological stimulant of acid secretion, a decrease in intragastric acidity induced by *H. pylori* infection might precede the gastrin release.

4.2.2.1 Acute Phase of *H. pylori* Infection Causes Hypochlorhydria

In the acute phase of *H. pylori* infection, transient hypochlorhydria occurs [3], which continues from several weeks to months [19–25]. Low acid secretion by acute *H. pylori* infection was demonstrated in the absence of loss of parietal cells [23], impaired permeability of gastric mucosa [23], and glandular atrophy [18]. Moreover, IL-1β which is produced by neutrophils and inhibits acid secretion was not involved in this stage. Instead, this low acid secretion in the acute stage is likely to result from direct contact parietal cells by *H. pylori* or its product [26, 27] and indirectly by activating intramural CGRP sensory neurons coupled to the stimulation of somatostatin and the inhibition of histamine secretion [14]. Studies about the ultrastructure of the stomach reported that *H. pylori* was observed adjacent to parietal cells and even detected in secretory canaliculi of these cells [28, 29]. In human, *H. pylori* infection also suppressed acid secretion via histamine, acetylcholine, and cAMP [30, 31], and this inhibition was resolved soon after the eradication of *H. pylori* [32]. This transient inhibition of acid secretion in the acute phase facilitates the successful settlement of *H. pylori* in the stomach.

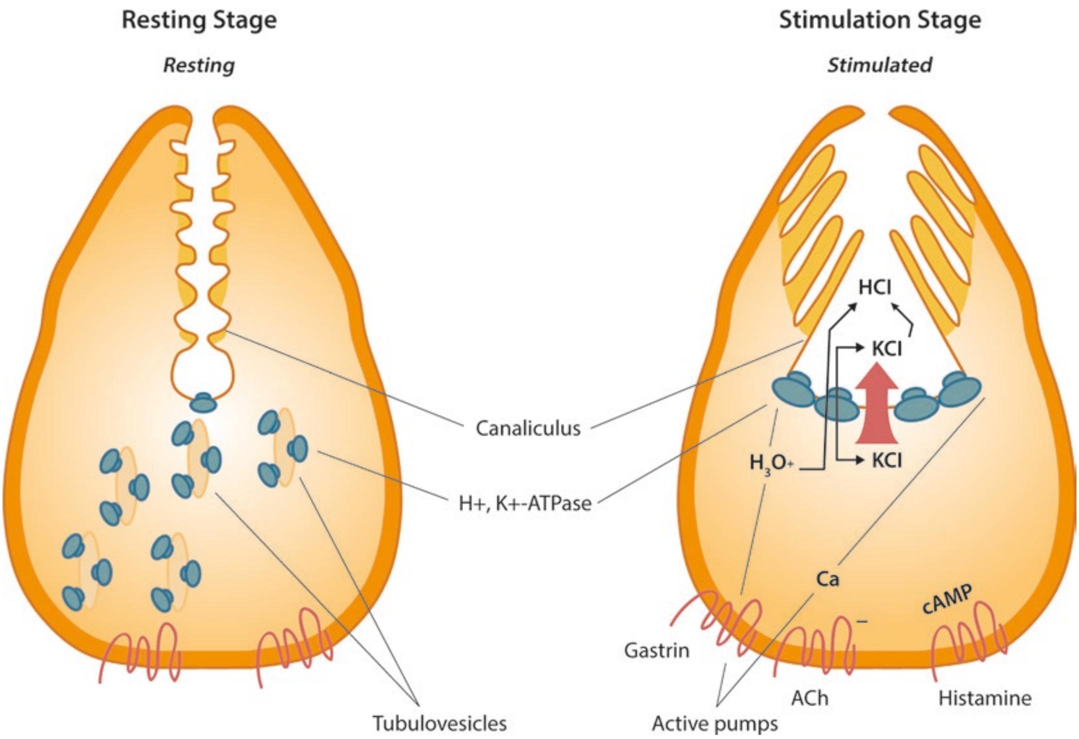

Fig. 4.4 Parietal cell structure of the resting and stimulated stage. In the resting parietal cell, most of the H^+, K^+-ATPase is sequestered in cytoplasmic tubulovesicles, and upon stimulation; it is trafficked to the apical microvilli of the secretory canaliculus (Adapted from Valle [9])

4.2.2.2 Effect of *H. pylori* Infection on H^+, K^+-ATPase

H. pylori-infected gastric mucosa or gastric epithelial cell lines showed the inhibition of HKα promoter activity in the endogenous or transfected H^+, K^+-ATPase [3]. Specifically, *H. pylori* inserts its protein into gastric cells through the type IV secretion system (T4SS), which looks like a cylindrical channel, and this inserted protein upregulated the nuclear factor (NF)-κB (Fig. 4.2). Interestingly, the site at which NF-κB combined in the promoter of H^+, K^+-ATPase was identified, and this fusion of the NF-κB p50 homodimer resulted in the repression of the transcription of HKα [3].

CagA protein encoded by *cag* pathogenicity island (*cag* PAI) and CagL, CagE, and CagM, which consist of the T4SS, and lytic transglycosylase are mechanistically involved in NF-κB activation and repression of HKα transcription (Fig. 4.2). CagL, a T4SS pilus component, binds to the integrin α5β1 to mediate translocation of virulence factors into the host cell and initiate signaling. During acute *H. pylori* infection, CagL dissociates ADAM17 (a disintegrin and a metalloprotease 17) from the integrin α5β1 complex and stimulates the ADAM17-dependent release of the heparin-binding epidermal growth factor (HB-EGF), EGF receptor (EGFR) stimulation, ERK1/2 kinase activation, and NF-κB-mediated repression of HKα [3] (Fig. 4.2).

4.2.2.3 Interaction Between *H. pylori* Infection and Gastric Acid Secretion Determining the Pattern of Gastritis

The interaction between *H. pylori* and gastric acid secretion also determines the pattern of *H. pylori*-induced gastritis (Fig. 4.5), which is a well-known and also very important concept regarding the final outcome of *H. pylori* infection such as duodenal ulcer (DU) or gastric cancer

(GC). That is, progression to certain disease by *H. pylori* infection is known to be determined by the degree of acid secretion when the organism invades the stomach. In the case of subjects with high acid secretion, *H. pylori* escapes from the corpus and settles in the antrum leading to antral-predominant gastritis with excessive gastrin release (Fig. 4.5). By contrast, when the organism comes into the subjects with low acid secretion, it migrates into the corpus with adequate acidity. This subsequently causes atrophy of parietal cells and aggravates hypochlorhydria. When *H. pylori* succeeds in the colonization, destroyed acid homeostasis influences the distribution of *H. pylori* in the stomach itself and the degree of gastritis. That is, *H. pylori* infection could diminish the number or function of D cells in the antrum, while it elevates the gastrin secretion of G cells via IL-8, IL-1β, or TNF-α [33, 34]. This disorganized endocrine system dumps an excessive amount of acid into the duodenum leading to DU (Fig. 4.5). On the other hand, the upregulation of IL-1β and TNF-α by *H. pylori* infection strongly suppresses acid secretion [15] and, at the same time, IL-1β reduces the secretion of histamine from ECL cells [35]. This makes *H. pylori* thrive in the corpus and destroy parietal cells, thereby finally aggravating impaired acid secretion. *H. pylori* infection, simultaneously, enhances the gastrin release. Consequently, the gastrin level in the blood was further accelerated due to reduced somatostatin secretion since IL-1β and TNF-α inhibit somatostatin release during the Th1 immune response [36] (Fig. 4.6). This chronic low acidic milieu accompanying atrophy and elevated gastrin has been considered to be a good condition for developing GC (Fig. 4.5).

4.2.2.4 Chronic Phase of *H. pylori* Infection and Gastric Acid Secretion

As mentioned above, the effect of *H. pylori* on acid secretion depends on the stage of infection or predominant location where the infection

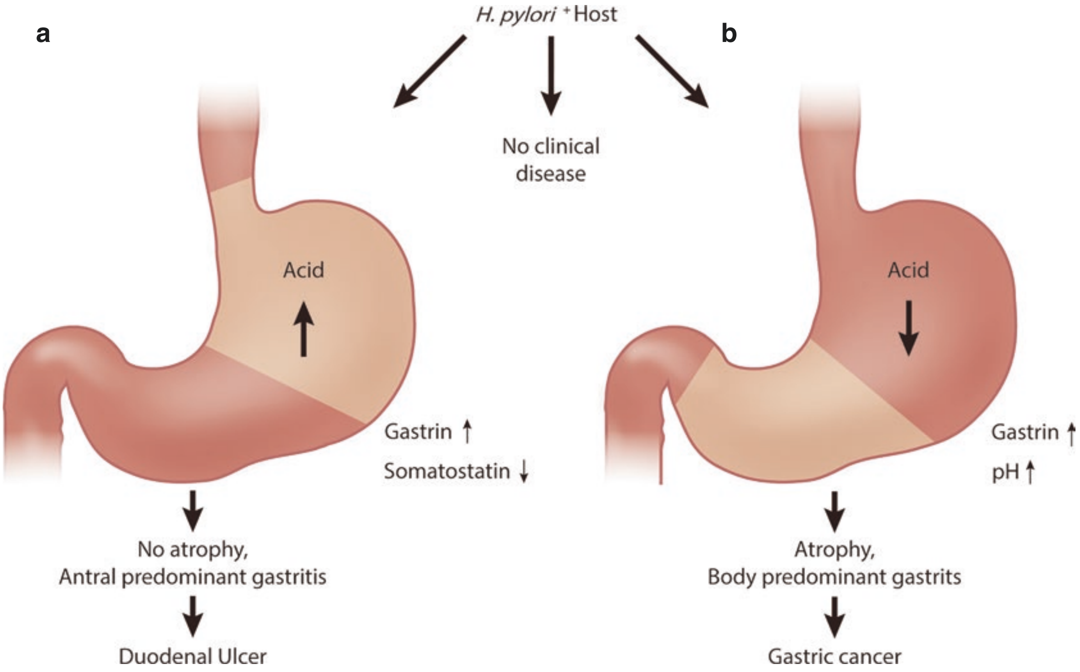

Fig. 4.5 Two patterns of *H. pylori*-induced gastritis. In the case of subjects with high acid secretion, *H. pylori* escapes from the corpus and settles in the antrum leading to antral-predominant gastritis with excessive gastrin release (**a**). By contrast, when the organism comes into the subjects with low acid secretion, it migrates into the corpus with adequate acidity (**b**). This subsequently causes atrophy of parietal cells and aggravates hypochlorhydria

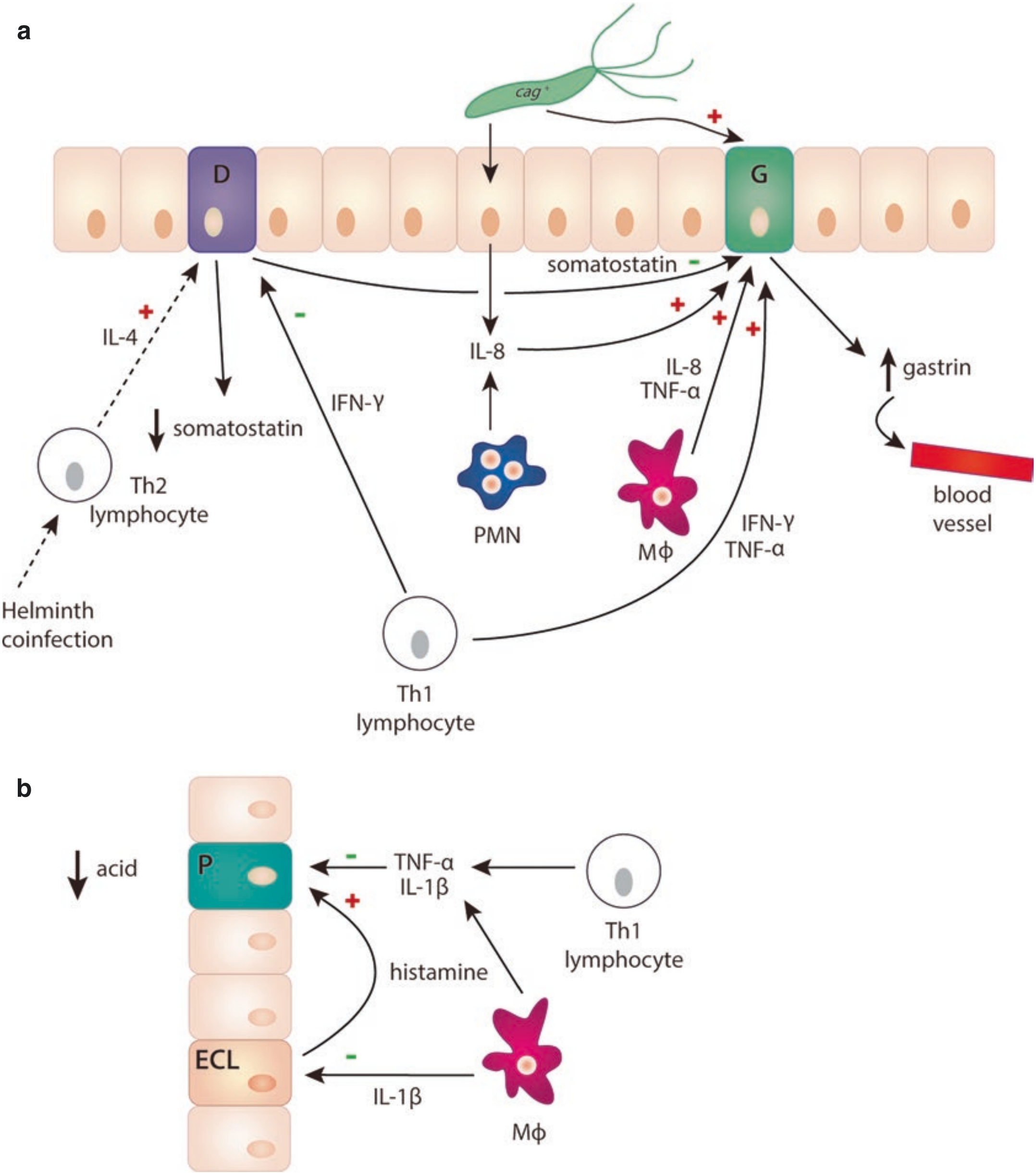
a
cag+
+
D
G
somatostatin
-
IL-4
+
-
+
+
+
IL-8
IFN-Y
IL-8
TNF-α
gastrin
somatostatin
Th2
lymphocyte
PMN
Mϕ
IFN-Y
TNF-α
blood
vessel
Helminth
coinfection
Th1
lymphocyte
b
acid
P
-
TNF-α
IL-1β
+
Th1
lymphocyte
histamine
ECL
-
IL-1β
Mϕ

Fig. 4.6 Cytokine-mediated alterations in gastrin and somatostatin secretion (**a**) and enterochromaffin-like (*ECL*) and parietal (*P*) cell function (**b**) in *H. pylori* infection. (**a**) IFN-γ from T helper (Th)1 cell, IL-8, and TNF-α stimulate gastrin secretion from G cells. In contrast, IFN-γ inhibits somatostatin release from D cells, decreasing its inhibitory effect on gastrin. IL-4 stimulates somatostatin secretion, which may be a potential mechanism by which helminth coinfection, and stimulation of Th2 responses, can protect against the development of corpus atrophy. (**b**) IL-1β suppresses ECL cell histamine release and IL-1β and TNF-α inhibit acid secretion from parietal cells. *PMN* polymorphonuclear cell (Adapted from Peek and Crabtree [36])

occurs. Chronic infection in the antrum causes the upregulation of gastrin and subsequent elevated acid secretion, while chronic infection in the corpus leads to impaired acid secretion by direct suppression of H^+, K^+-ATPase or the involvement of cytokines such as IL-1β or TNF-α. Therefore, the direct inhibition of H^+, K^+-ATPase, indirect inhibition through cytokines, and loss of parietal cells by ongoing inflammation were three mechanisms for the low acid secretion in the chronic corpus-predominant gastritis.

4.2.2.5 Gastrin and Somatostatin in Regard to *H. pylori* Infection

Gastrin release is stimulated by extramural cholinergic and intramural cholinergic and noncholinergic factors and inhibited by somatostatin [37] (Fig. 4.7). Somatostatin release is stimulated by luminal (acid), paracrine, and hormonal factors (gastrin) and by intramural noncholinergic factors and is inhibited by extramural cholinergic factors. Levi et al. [38] reported that both basal/stimulated acid secretion and basal/meal-stimulated plasma gastrin levels were significantly higher in *H. pylori*-positive DU patients ($n = 25$) compared with *H. pylori*-negative DU patients ($n = 6$), and this phenomenon has been named as "the gastrin link." Their data clearly demonstrated that *H. pylori* infection induced hypergastrinemia that was followed by an increase in acid secretion in DU patients (Fig. 4.5). Consequently, the eradication of *H. pylori* reduced the output of gastrin in the stomach [39].

In mammals, the gastrointestinal (GI) tract and pancreas contain the largest amounts of somatostatin. Most of the GI somatostatin immunoreactivity is confined to the mucosal layer [40], where epithelial endocrine cells and D cells are

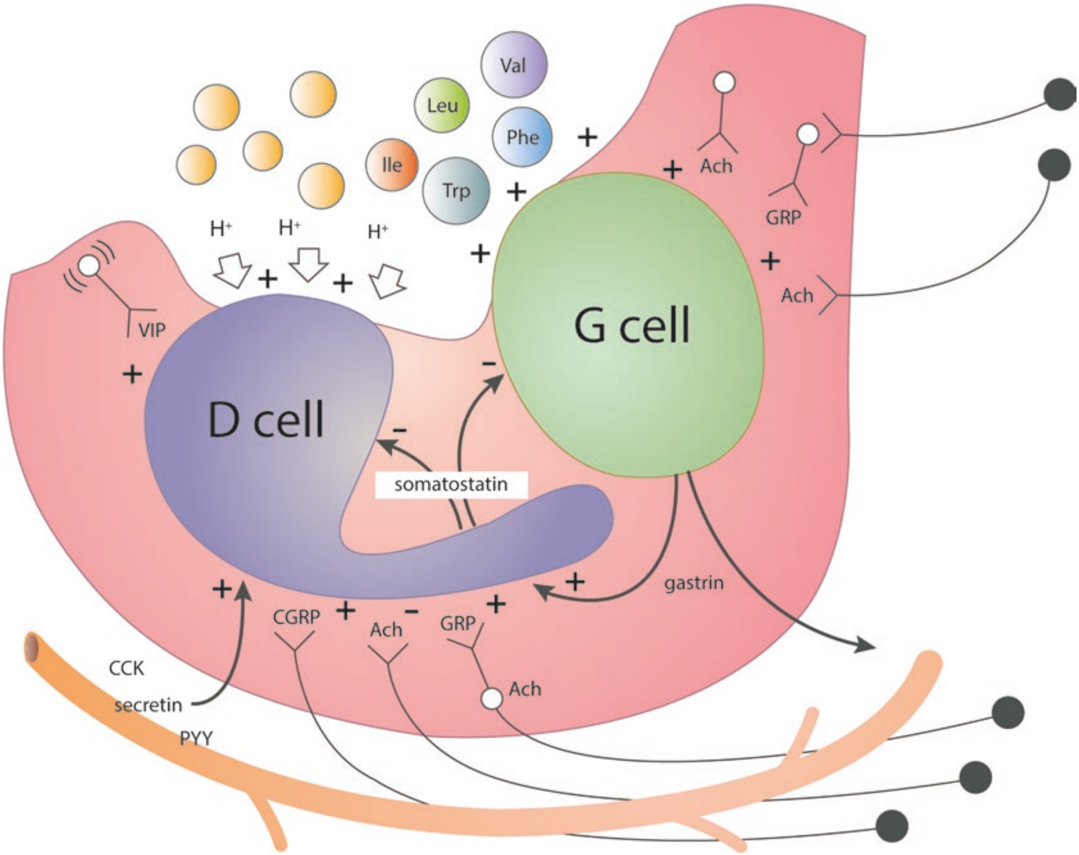

Fig. 4.7 Gastrin release is stimulated by extramural cholinergic and intramural cholinergic and noncholinergic factors and inhibited by somatostatin. Somatostatin release is stimulated by luminal (acid), paracrine, and hormonal factors (gastrin) and by intramural noncholinergic factors and is inhibited by extramural cholinergic factors. *CCK* cholecystokinin, *PYY* peptide YY, *CGRP* calcitonin gene-related peptide, *VIP* vasoactive intestinal peptide, *GRP* gastrin-releasing peptide, *Ach* acetylcholine (Adapted from Kaneko et al. [37])

localized. In the antrum, the D cells have apical membranes that are exposed to the lumen ("open cells"). In the corpus, the D cells are of the "closed" type; they are not exposed to the luminal surface of the mucosa [41]. Somatostatin plays an important role in the regulation of gastric acid secretion by inhibiting gastrin release. In the antral mucosa, the open D cell releases somatostatin in response to increased acidity in the gastric lumen. As the apical surface of D cells opens onto the gastric lumen, changes in pH may be sensed directly through chemoreceptors on the apical membranes.

The effect of *H. pylori* infection on gastric levels of somatostatin has been relatively well investigated. Antral somatostatin concentrations were decreased in *H. pylori*-infected patients, but not the corpus [42] which have been demonstrated by measuring the somatostatin concentration in the biopsy specimens from both sites [43, 44]. Moss et al. [45] demonstrated that the eradication of *H. pylori* from patients with DU caused an approximately two-fold increase in somatostatin mRNA in antral, but not in corpus biopsies. They also showed an increase in D cell numbers after *H. pylori* eradication in subjects with active DU [45].

4.2.3 Effect of *H. pylori* Eradication on Gastric Acid Secretion

The effect of *H. pylori* eradication on gastric acid secretion also starts from the gastritis pattern such as antral- or corpus-predominant gastritis because the ongoing cascade of gastritis and gastric acid secretion caused by *H. pylori* will stop right away. It will also cause an effect on the gastrin, somatostatin, and other gastric hormones. Basically, the changes of acid secretion after *H. pylori* eradication depend on the degree of inflammation in the corpus where parietal cells localize. More specifically, in the setting of antral-predominant gastritis with intact corporal glands, *H. pylori* eradication reduces gastrin and subsequently acid secretion, as well. In contrast, in the setting of severe corporal inflammation, the inhibition of parietal cells by *H. pylori* disappears, leading to the elevated acid secretion [36].

Several studies reported the immediate increase in acid secretion after the administration of anti-*H. pylori* agents [32, 36]. These results came from the termination of direct contact of parietal cells with *H. pylori* or its products [26, 27] and reduction of IL-1β or TNF-α which represses acid secretion. There was a question regarding the long-term change of acid secretion after *H. pylori* eradication, especially, for the relationship with the reversibility of atrophic gastritis in the corpus. Osawa et al. [46] performed a study on 111 subjects with chronic gastritis under the GC surveillance program and reported that the mRNA of H^+, K^+-ATPase increased 12 weeks after *H. pylori* eradication even in severe atrophy without changes in the number of parietal cells. That is, median mRNA expression levels of H^+, K^+-ATPase in the gastric mucosa increased 250-fold after *H. pylori* eradication accompanied by the attenuation of IL-1β. A large increase in H^+, K^+-ATPase expression was observed even in patients with severe atrophic gastritis. In contrast, fold increases in mRNA expression levels, including intrinsic factor, anion exchanger 2, and M3 muscarinic receptor which are involved in acid secretion export in the basal and apical sides of parietal cells, after eradication therapy, were limited to 1.4, 2.3, and 2.5 times, respectively [46]. These results that gastric H^+, K^+-ATPase mRNA expression was markedly restored 12 weeks after successful *H. pylori* eradication suggests a central role of the restoration of H^+, K^+-ATPase expression in gastric acid secretion recovery after *H. pylori* eradication in the absence of alteration of parietal cell number [46]. In addition, El-Omar et al. [47] reported that hypochlorhydria seen in patients with mild degrees of body atrophy improved readily after *H. pylori* eradication. As body gastritis preceded the development of body atrophy by decades in *H. pylori* infection [17, 48], the duration of the infection, or age of the patients in many cases, could be considered to be a principal determinant for the reversibility of acid secretion by *H. pylori* eradication. Similarly, when Iijima et al. [49] assessed gastrin-stimulated acid output and histologic evaluation of biopsy specimens prior to and 1.7 months after eradication and gastric acid secretion over 5 years after *H. pylori* eradication in 12 patients with hypochlorhydria (<0.6 mmol/10 min), gastric acid secretion was reversed to the normal range in nine of 23 patients (39 %) at 7 months after eradication [49]. In the long-term follow-up, gradual and significant recovery in gastric acid secretion was observed up to 2 years post-therapy [49]. However, there was no additional increase during the last 3 years of 5-year follow-up period, leaving the acid secretory levels subnormal in the majority of the patients [49]. This long-term follow-up study suggests that the pathologic process has already progressed to an irreversible stage in the majority of *H. pylori*-positive patients with marked body atrophy and profound hypochlorhydria [49]. However, further long-term studies are needed in the various clinical situations in the future as atrophy and intestinal metaplasia showed reversible changes with *H. pylori* eradication in some of the persons even after a 5-year follow-up period [50, 51].

4.3 Ghrelin

Ghrelin is a 28-amino acid peptide hormone, primarily produced and secreted from the gastric mucosa [52]. The stomach is considered as the major source of circulating ghrelin since severe reduction in blood ghrelin levels is observed in patients who had undergone gastrectomy [53]. Ghrelin is produced in oxyntic cells that are prominent in the corpus of the stomach, and ghrelin-producing cells are located from the base to the neck of the glands. It is also secreted from the small intestine and the colon [54] and is also expressed in the hypothalamus [55], the pituitary [56], and several tissues in the periphery [57]. Although the major active product of the ghrelin gene is the 28-amino acid peptide acylated at Ser^3 with C8:0, recent studies have revealed that the ghrelin gene can generate various molecules besides ghrelin, which include des-acyl ghrelin [58]. The human ghrelin gene, located on the short arm of chromosome 3 (3p25-26), is composed of five exons and four introns [59] (Fig. 4.8). The main ghrelin mRNA transcript in human codes for a 117-amino acid long peptide, prepro-ghrelin (1–117). The signal peptide sequence, prepro-ghrelin (1–23) of prepro-ghrelin (1–117), is cleaved to form pro-ghrelin (1–94) (Fig. 4.8). A series of posttranslational steps, including the process of protease cleavage and acyl modification of the ghrelin precursor peptide, results in the production of mature ghrelin peptides (acyl and des-acyl ghrelin) or other ghrelin gene-associated peptides (C-ghrelin and obestatin) (Fig. 4.8). The ghrelin peptide is acylated by the enzyme ghrelin O-acyl transferase (GOAT) [60], which is expressed predominantly in the stomach, gut, and pancreas, but also at other sites [61]. This acyl modification of ghrelin is easily cleaved during sample extraction. Thus, acyl ghrelin should be isolated from blood specimens by adding ethylenediaminetetraacetic acid (EDTA) with aprotinin or *p*-hydroxymecuribenzoic acid. That is, the plasma should be separated by centrifugation and immediate acidification before freezing at −80 °C to ensure the stability of acyl ghrelin during storage. The nonacylated form of ghrelin without octanoic acid modification at the Ser^3 residue, des-acyl ghrelin, is also present at a significant level in both the stomach and blood [58, 62]. Des-acyl ghrelin is the most abundant ghrelin-related molecule in the body, comprising 80–90 % of the total circulating ghrelin, and has a longer half-life.

4.3.1 Role of Ghrelin

In humans, ghrelin stimulates gastric motility [63] and acid secretion [64]. Acyl ghrelin has been identified as the endogenous ligand of the growth hormone secretagogue receptor (GHS-R) [52]. Acyl ghrelin acts in the GHS-R on vagal afferent nerve fibers in the stomach [65], which transmit this signal to the nucleus of the solitary tract (NTS) (Fig. 4.9). From the NTS, the information is projected to the arcuate nucleus (ARC) of the hypothalamus, where neuropeptide Y (NPY) neurons are activated (Fig. 4.9). The NPYY2 and/or Y4 receptor in the CNS may be involved in the upper GI motility because Y2 and

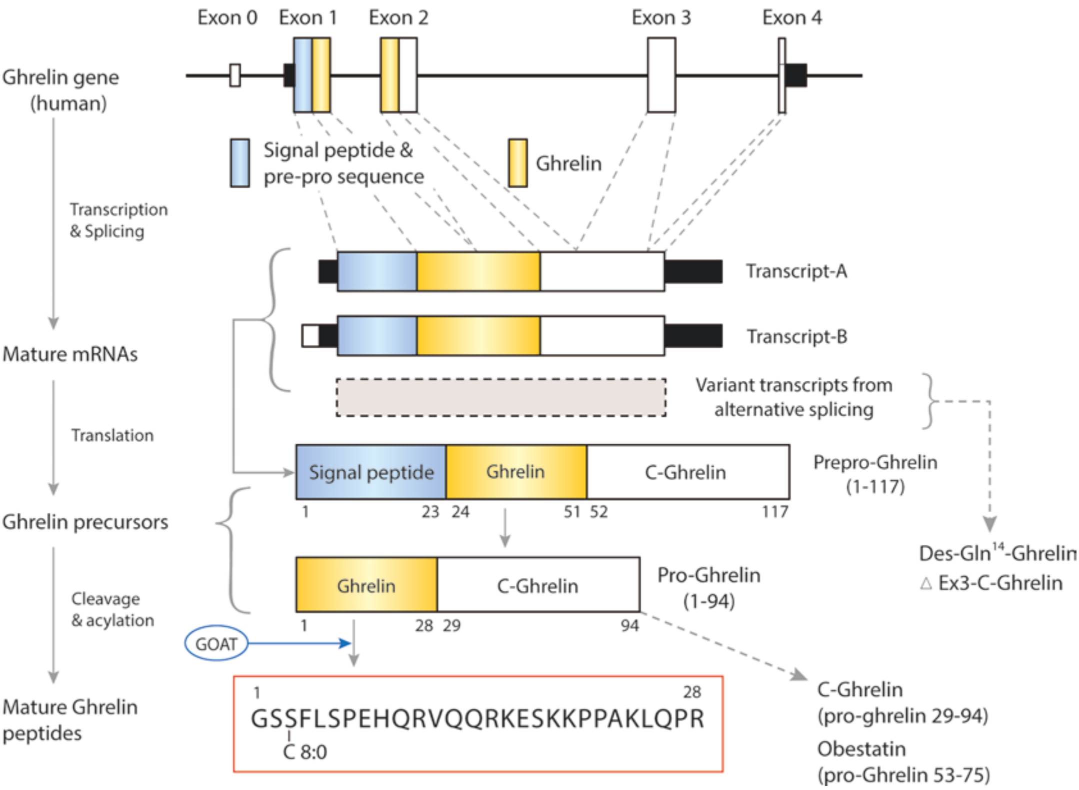
Exon 0
Exon 1
Exon 2
Exon 3
Exon 4
Ghrelin gene (human)
Signal peptide & pre-pro sequence
Ghrelin
Transcription & Splicing
Transcript-A
Transcript-B
Mature mRNAs
Variant transcripts from alternative splicing
Translation
Signal peptide
Ghrelin
C-Ghrelin
Prepro-Ghrelin (1-117)
1
23 24
51 52
117
Ghrelin precursors
Des-Gln14-Ghrelin
△ Ex3-C-Ghrelin
Cleavage & acylation
Ghrelin
C-Ghrelin
Pro-Ghrelin (1-94)
1
28 29
94
GOAT
Mature Ghrelin peptides
1
28
GSSFLSPEHQRVQQRKESKKPPAKLQPR
C 8:0
C-Ghrelin (pro-ghrelin 29-94)
Obestatin (pro-Ghrelin 53-75)

Fig. 4.8 Structure of the human ghrelin gene and processing steps from the ghrelin gene to acyl ghrelin, des-acyl ghrelin, or other ghrelin-associated peptides. The human ghrelin gene is composed of five exons and four introns. The major mRNA transcript (transcript-A) of the ghrelin gene is translated into a 117-amino acid ghrelin precursor, prepro-ghrelin (1–117). The signal peptide sequence, prepro-ghrelin (1–23) of prepro-ghrelin (1–117), is cleaved to form pro-ghrelin (1–94). A series of posttranslational steps, including the process of protease cleavage and acyl modification of the ghrelin precursor peptide, results in the production of mature ghrelin peptides (acyl and des-acyl ghrelin) or other ghrelin gene-associated peptides (C-ghrelin and obestatin). Besides these peptides, several other ghrelin gene-derived peptides with or without acyl modification (des-Gln14-ghrelin, ΔEx3-C-ghrelin, etc.) are predicted to be produced from the splicing variants of the ghrelin gene transcripts. *GOAT* ghrelin-O-acyltransferase (Modified from Nishi et al. [59])

Y4 receptor agonists can induce phase III-like contractions in the duodenum when given to animals in the fed state [66]. From the ARC, the signal is finally transmitted to the dorsal motor nucleus of the vagus nerve ((DVC) dorsal vagal complex) and via vagal efferent fibers, and fasted motor activity is induced in the gut [67] (Fig. 4.9). Acyl ghrelin is considered as a short regulator of food intake in both animals and humans [68, 69]. In rats, acute and chronic administration of ghrelin enhances food intake and weight gain [70, 71]. Peripheral administration of ghrelin produced a 28% increase of food intake in normal-weight healthy volunteers [68]. The subjects who received exogenous ghrelin reported an increase in appetite and showed a higher caloric intake after placebo [72]. This enhanced motor activity of the GI tract has been considered to play a role of mechanical cleansing of the stomach and the intestine in preparation for the next meal. In healthy volunteers, the peripheral administration of ghrelin induces the occurrence of phase III of the migrating motor complex about 20 min after administration of ghrelin [73]. Moreover, it induces a premature phase III originating in the stomach about 14 min after its injection [73]. A positive correlation was reported between preprandial ghrelin concentration and gastric emptying time.

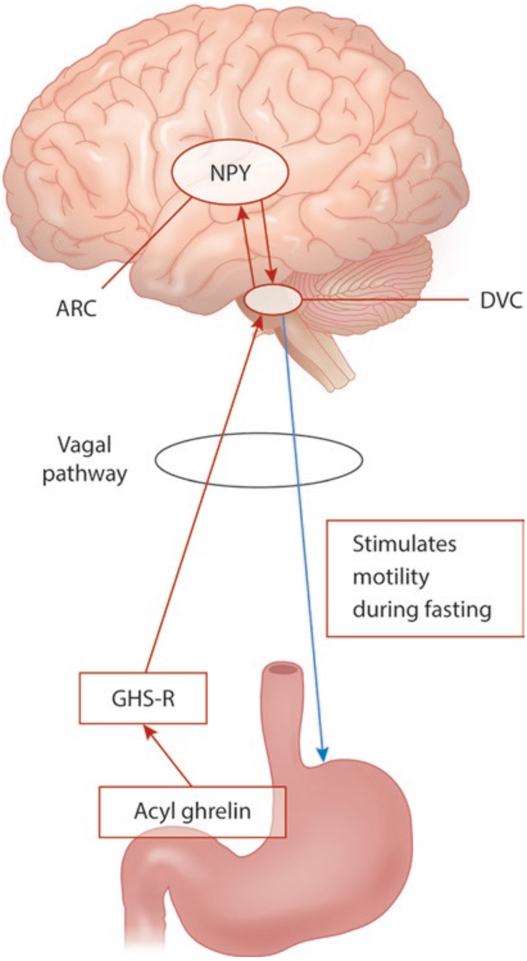

Fig. 4.9 The effects of acyl ghrelin via the brain-gut axis. *ARC* arcuate nucleus, *DVC* dorsal vagal complex, *GHS-R* growth hormone secretagogue receptor, *NPY* neuropeptide Y (Modified from Fujimiya et al. [66])

4.3.2 Regulation of Ghrelin in Regard to *H. pylori* Infection

Since ghrelin is predominantly produced in the stomach, many researchers evaluated the effect of *H. pylori* infection on ghrelin expression in the gastric mucosa and blood. Theoretically, *H. pylori*-related gastritis may progress to atrophy with the loss of oxyntic glands leading to a negative effect on ghrelin production in the stomach. Isomoto et al. [74] well demonstrated that the gastric ghrelin mRNA expression level in *H. pylori*-positive patients was significantly lower than that in *H. pylori*-negative subjects, and a similar trend was noted for ghrelin peptide contents. In addition, plasma ghrelin concentrations in *H. pylori*-infected patients were significantly lower than those in uninfected subjects and increased following cure of the infection [74]. Furthermore, plasma ghrelin levels correlated positively with the expression levels of ghrelin mRNA and peptide products. There was a significant stepwise decrease in the gastric ghrelin mRNA expression, peptide contents, and density of ghrelin immunoreactive cells with progression of histologic severity of glandular atrophy in the corpus [74]. However, the effect of *H. pylori* on the circulating level of ghrelin is not simple mainly due to the complicated metabolism pathway of ghrelin. That is, the circulating level of ghrelin is determined by the balance among its rate of secretion, degradation, and clearance. While plasma esterases deacylate acyl ghrelin, plasma proteases degrade circulating ghrelin. Consequently, studies that have compared blood ghrelin levels between *H. pylori*-infected and noninfected subjects showed inconsistent results [75–97] (Table 4.1). More basically, there are several factors such as gastrin, insulin-like growth factor (IGF) 1, obesity, insulin resistance, hyperinsulinemia, and cholesterol and urine excretion in addition to *H. pylori* infection that could affect plasma ghrelin concentration. However, Nweneka and Prentice [98] concluded that circulating ghrelin was significantly lower in *H. pylori*-positive subjects in a meta-analysis. The effect of *H. pylori* on ghrelin production has also been related to *H. pylori* virulence [1]. Patients with type I strain *H. pylori*, expressing CagA and VacA, have lower circulating ghrelin levels than those with less virulent type II strain [99]. Probably, the extent of gastric damage and the duration of the infection play a key role in the modulation of ghrelin levels by *H. pylori* [100]. Similar data have also been reported in patients with autoimmune gastritis [101].

Table 4.1 Studies that compared circulating levels of ghrelin between *H. pylori*-positive and *H. pylori*-negative subjects

Author	Subjects	Sex	Age	No	Method	Specimen	Ghrl type	Results in HP (+)
Gokcel et al. (2003), Turkey [75]	Not stated	W	A	39	Commercial EIA	Plasma	Total	→
Isomoto et al. (2004), Japan [76]	Sick	B	A	68	Commercial RIA	Plasma	Total	↓
Isomoto et al. (2005), Japan [74]	Dyspepsia	B	A	81	In-house RIA	Plasma	Total	↓
Isomoto et al. (2005), Japan [77]	Sick	B	A	89	In-house RIA	Plasma	Total	↓
Osawa et al. (2005), Japan [78]	Healthy (health checkup)	M	A	160	In-house RIA	Plasma	Total	↓
Shiotani et al. (2005), Japan [79]	Healthy	B	A	132	Commercial ELISA	Serum	Total	↓
Konturek et al. (2006), Poland [80]	Healthy	B	B	180	Human RIA	Serum	Total	↓
Plonka et al. (2006), Poland [81]	Healthy	B	B	538	Commercial RIA	Serum	Total	↓
Plonka et al. (2006), Poland [82]	Healthy	B	C	287	Commercial RIA	Serum	Total	↓
Salles et al. (2006), France [83]	Hospitalized in geriatric	B	A	62	Commercial RIA	Plasma	Total	↓
Alonso et al. (2007), Spain [84]	Type I DM	B	A	15	Commercial RIA	Plasma	Total	↓
An et al. (2007), Korea [85]	Gastric cancer	B	A	41	Commercial ELISA	Plasma	Total	→
Cindoruk et al. (2007), Turkey [86]	Sick (normal 24 pH without atrophy)	B	A	50	RIA	Plasma	Total	→
D’Onghia et al. (2007), Italy [87]	Colon cancer	B	A	29	RIA	Serum	Total	↓
de Martel (2007), USA [88]	Healthy + sick	B	A	110	Commercial ELISA	Serum	Total	→
Jun et al. (2007), Korea [89]	CG	B	A	63	Commercial RIA	Plasma	Total	→
Pacifico et al. (2008), Italy [90]	Healthy + GI symptom	B	C	85	Commercial RIA	Serum	Total	→

Roper et al. (2008), USA [91]	Healthy	M	A	256	Commercial EIA	Serum	Total	→
Shak et al. (2008), USA [92]	BMI >35 kg/m^2	B	A	24	Commercial EIA	Plasma	Total and acyl	→
Chuang et al. (2009), Taiwan [93][a]	PU + FD	M	A	145	Commercial RIA	Plasma	Total	↓
Chuang et al. (2009), Taiwan [93][a]	PU + FD	W	A	196	Commercial RIA	Plasma	Total	→
Gao et al. (2009), China [94]	Healthy (health checkup)	B	A	100	Commercial RIA	Plasma	Total	↓
Kawashima et al. (2009), Japan [95]	Healthy + sick (PU + CG)	B	A	220	Commercial EIA	Plasma	Acyl	↓
Campana et al. (2009), Italy [96]	Healthy + CG (HP(−))	B	A	50	RIA	Plasma	Acyl	↑ in atrophy
Choi et al (2016), Korea [97]	Healthy + FD	B	A	282	Commercial ELISA	Plasma	Acyl	↓

↑ higher, ↓ lower, → no significant difference

A adults, *B* both, *C* child, *W* women, *M* men, *Ghrl* ghrelin, *HP H. pylori*, *No* sample size, *CG* chronic gastritis, *DM* diabetes mellitus, *FD* functional dyspepsia, *PU* peptic ulcer, *Sick* patients who visited clinics, but the specific condition is not stated, *EIA* enzyme immunoassay, *ELISA* enzyme-linked immunosorbent assay, *RIA* radioimmunoassay, *RT-PCR* real-time polymerase chain reaction

[a] Articles written in English and assay method were stated

4.3.3 Effect of Eradication of *H. pylori* on Ghrelin

Three studies demonstrated that *H. pylori* eradication increases ghrelin mRNA [102–104]. Osawa et al. [104] demonstrated that median prepro-ghrelin mRNA expression was increased nearly fourfold at the point of 12 weeks after *H. pylori* eradication in 134 subjects. The ghrelin-immunoreactive cell number/mm^2 also increased in 50 *H. pylori*-eradicated patients in the absence of a significant change of atrophy and intestinal metaplasia [105]. However, Choe et al. [106] failed to demonstrate a change in ghrelin expression 4 weeks after *H. pylori* eradication. The effect of *H. pylori* eradication on the circulating level of ghrelin was more evaluated than on the gastric levels but the results are still inconsistent (Table 4.2). At first, Nwokolo et al. [107] reported an increase in circulating plasma ghrelin levels following eradication of *H. pylori* in their study of 12 healthy subjects. Further studies reported elevated levels of blood ghrelin after the cure of *H. pylori* [95, 103, 108]. However, subsequent studies found no change [74, 76, 77, 86, 102, 106] or even a decrease [90, 105] in circulating ghrelin levels. When Nweneka and Prentice [98] performed a meta-analysis, *H. pylori* eradication has no effect on circulating ghrelin levels. Nevertheless, pre-eradication elevation of ghrelin may be a predictor of a fall in plasma levels post-eradication [78, 98]. Regarding the relationship between ghrelin and functional dyspepsia (FD) in terms of *H. pylori* infection, two studies reported that fasting total ghrelin levels were significantly lower in patients with dysmotility-like FD than healthy volunteers [109, 110]. In addition, Shindo et al. [111] also showed significantly lower plasma acyl ghrelin levels in patients with postprandial distress syndrome (PDS) compared with healthy volunteers. In our study, the plasma acyl ghrelin level was lower in the PDS group than in the control and epigastric pain syndrome (EPS) group [97]. One year after the eradication of *H. pylori*, the plasma acyl ghrelin level and gastric prepro-ghrelin mRNA expression were increased in the PDS type ($p = 0.004$ and $p < 0.001$, restrictively) [97]. In addition, patients with symptomatic improvement demonstrated an increase in plasma acyl ghrelin (11.51–21.00, $p = 0.040$). From these results we concluded that the plasma acyl ghrelin plays a role in the development of PDS, and *H. pylori* eradication upregulates prepro-ghrelin mRNA and plasma acyl ghrelin, contributing to the improvement in PDS symptoms [97]. However, usually there is a difficulty in enrolling enough numbers of follow-up cases. Thus, further studies are necessary to identify the true effect of *H. pylori* eradication on the gastric and circulating ghrelin levels as well as on the symptom changes in the patients with FD.

Table 4.2 Studies that compared the circulating levels of ghrelin before and after *H. pylori* eradication

Authors	Subjects	Sex	Age	No	Method	Specimen	Ghrl type	FU (weeks)	Results after eradication
Nwokolo et al. (2003), UK [107]	Healthy	B	A	10	Commercial RIA	Plasma	Total	6	↑
Isomoto et al. (2004), Japan [76]	Sick	B	A	9	Commercial RIA	Plasma	Total	4	→
Isomoto et al. (2005), Japan [74]	Dyspepsia	B	A	43	In-house RIA	Plasma	Total	4	→
Isomoto et al. (2005), Japan [77]	Sick	B	A	10	In-house RIA	Plasma	Total	4	→
Osawa et al. (2006), Japan [104]	Healthy + PU/CG	M	A	134	In-house RIA	Plasma	Total	12	↓
Choe et al. (2007), Korea [106]	Sick	B	A	8	Commercial ELISA	Plasma	Total	4	→
Cindoruk et al. (2007), Turkey [86]	Sick (normal 24 pH without atrophy)	B	A	23	RIA	Plasma	Total	12	→
Czesnikiewicz-Guzik et al. (2007), Poland [108]	Sick	W	A	49	Commercial RIA	Plasma	Total	4	↑
Jang et al. (2008), Korea [103]	PU	B	A	16	Commercial RIA	Plasma	Total	NA	↑
Pacifico et al. (2008), Italy [90]	Healthy + sick (GI symptom)	B	C	22	RIA	Serum	Total	52	↓
Kawashima et al. (2009), Japan [95]	Both (PU + CG)	B	A	49	Commercial EIA	Plasma	Acyl	23	↑
Lee et al. (2010), Korea [102]	Healthy	B	A	9	ELISA	Plasma	Total	5	→
Choi et al (2016), Korea [97]	Healthy + FD	B	A	79	Commercial ELISA	Plasma	Acyl	67	↑

↑ increased, ↓ decreased, → no significant change

A adults, *B* both, *C* child, *W* women, *M* men, *Ghrl* ghrelin, *HP H. pylori*, *NA* not available, *No* sample size, *CG* chronic gastritis, *FU* follow-up, *GI* gastrointestinal, *PU* peptic ulcer, *FD* functional dyspepsia, *Sick* patients who visited clinics, but the specific condition is not stated, *EIA* enzyme immunoassay, *ELISA* enzyme-linked immunosorbent assay, *RIA* radioimmunoassay, *RT-PCR* real-time polymerase chain reaction

4.4 Leptin

Leptin was discovered in 1994 as a hormone produced by the adipose tissue with a modulatory effect on feeding behavior and weight control [112]. Leptin is a product of the obese (*ob*) gene, which is located on chromosome 7 in humans and acts through its receptor OB-R. Interestingly, the stomach has been identified as an important source of leptin [112], and leptin-producing cells were found to be localized in the lower half of the fundic glands, a site similar to that of the pepsinogen-secreting chief cells. The soluble isoform of its receptor (Ob-R) is secreted by chief cells in the gastric mucosa [113]. Moreover, intestines do express membrane-bound leptin receptors on their brush border [112–114] (Fig. 4.10). Collectively, gastric exocrine and endocrine secretions of leptin constitute a gastroenteric axis to coordinate its roles in the GI tract [115] (Fig. 4.11).

4.4.1 Regulation and Role of Gastric Leptin

Gastric leptin is sensitive to the nutritional status of the body. Fasting for 48 h induced a decrease in gastric leptin expression and content in rats [116]. Food intake quickly depletes gastric leptin, while sustained feeding stimulates the leptin gene expression and leptin synthesis [115, 116]. Leptin derived from the stomach can be distinguished from adipocyte leptin through its rapid increased secretion following a meal and through its exocrine secretion (i.e., mainly in the gastric lumen). While the gastric mucosa secretes leptin within minutes after the beginning of food intake, it takes several hours for adipocytes to release significant amounts of leptin. Differences in the time frame of secretion between the adipose tissue and gastric mucosa may reflect different roles. Gastric leptin remains stable in gastric juice even at pH 2 [117] which makes leptin to affect gastric functions. It has been considered that gastric leptin may exert paracrine effects within the gastric mucosa or stimulating vagal afferents to signal the central nervous system [113]. Gastric leptin controls food intake and satiety sensations by acting on the stomach itself. It potentiates the effect of cholecystokinin (CCK) by slowing gastric emptying and promoting gastric distension [118], without a direct effect on the CNS [119]. It also stimulates the production of glucagon-like peptides 1 and 2 (GLP1 and GLP2) which inhibit gastric emptying [120, 121].

Leptin receptor isoforms have been found in the rat nodose ganglion, which contains the cell bodies of vagal afferent neurons, and in the vagus nerve proper [122, 123]. Signals arising from the upper GI tract are conveyed by the viscerosensitive vagal afferent neurons to the nucleus of the solitary tract (NTS), and then to the hypothalamus where they participate in the process of meal-induced termination of food intake [115, 124] (Fig. 4.11). CCK secreted from duodenal endocrine I cells typically functions as one of these short-term satiety signals via the activation of the CCK-1 receptor [125]. In short, while adipose leptin acts on the long-term mainly through its interactions with the central nervous system, gastric leptin acts locally on the gastric mucosa for regulating food intake. Gastric leptin influences both the intestinal tract and the central nervous system [115] (Fig. 4.11).

4.4.2 Regulation of Leptin in Regard to *H. pylori* Infection

Briefly, the gastric mucosal damage due to *H. pylori* provokes a marked increase of leptin and gastrin levels, and a reduction in ghrelin plasma levels, and this phenomenon may contribute to the occurrence of dyspeptic symptoms and appetite alteration [1]. *H. pylori* infection significantly increased gastric leptin expression, and cure of the infection significantly reduced this expression with a concomitant increase in the body mass index (BMI) [126]. *H. pylori*-infected gastric mucosa was found to be capable of releasing larger amounts of leptin mRNA than that without *H. pylori* infection [89, 112]. Although majority of studies have shown an increase of gastric leptin mRNA in *H. pylori*-infected subjects (Table 4.3), still there are discrepancies [83, 91].

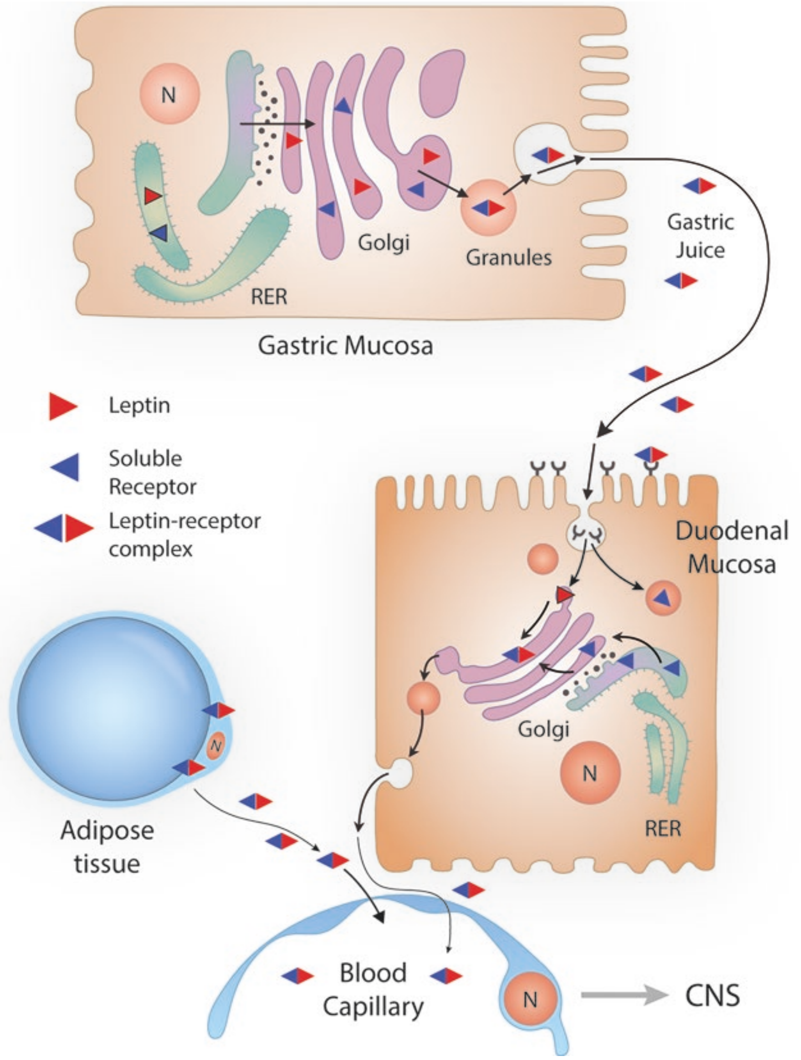
N
Golgi
Granules
RER
Gastric Juice
Gastric Mucosa
Leptin
Soluble Receptor
Leptin-receptor complex
Duodenal Mucosa
Golgi
N
RER
Adipose tissue
N
Blood Capillary
N
CNS

Fig. 4.10 Schematic drawing that illustrates the secretion of leptin by the adipocyte and the gastric chief cell. Both types of cells secrete the leptin receptor. Both the leptin and the leptin receptor are synthesized in the rough endoplasmic reticulum, transferred to the Golgi apparatus, and packaged into either small vesicles (adipocytes) or secretory granules (gastric cells). At the level of the trans-cisternae of the Golgi and in the secretory granules, leptin binds its soluble receptor to form the leptin–leptin receptor complex. This complex is discharged by both cell types through an exocytotic event. The adipose tissue secretes toward the blood circulation, while the gastric cells secrete in an exocrine fashion into the gastric juice. Leptin in the gastric juice is vehiculated to the duodenal lumen. In the duodenum the leptin–leptin receptor complex is internalized and separated. The leptin is channeled toward the trans-Golgi cisternae, and it binds a newly synthesized soluble leptin receptor. The complex reaches the blood circulation. *RER* rough endoplasmic reticulum, *CNS* central nervous system, *N* nucleus (Adapted from Cammisotto and Bendayan [114])

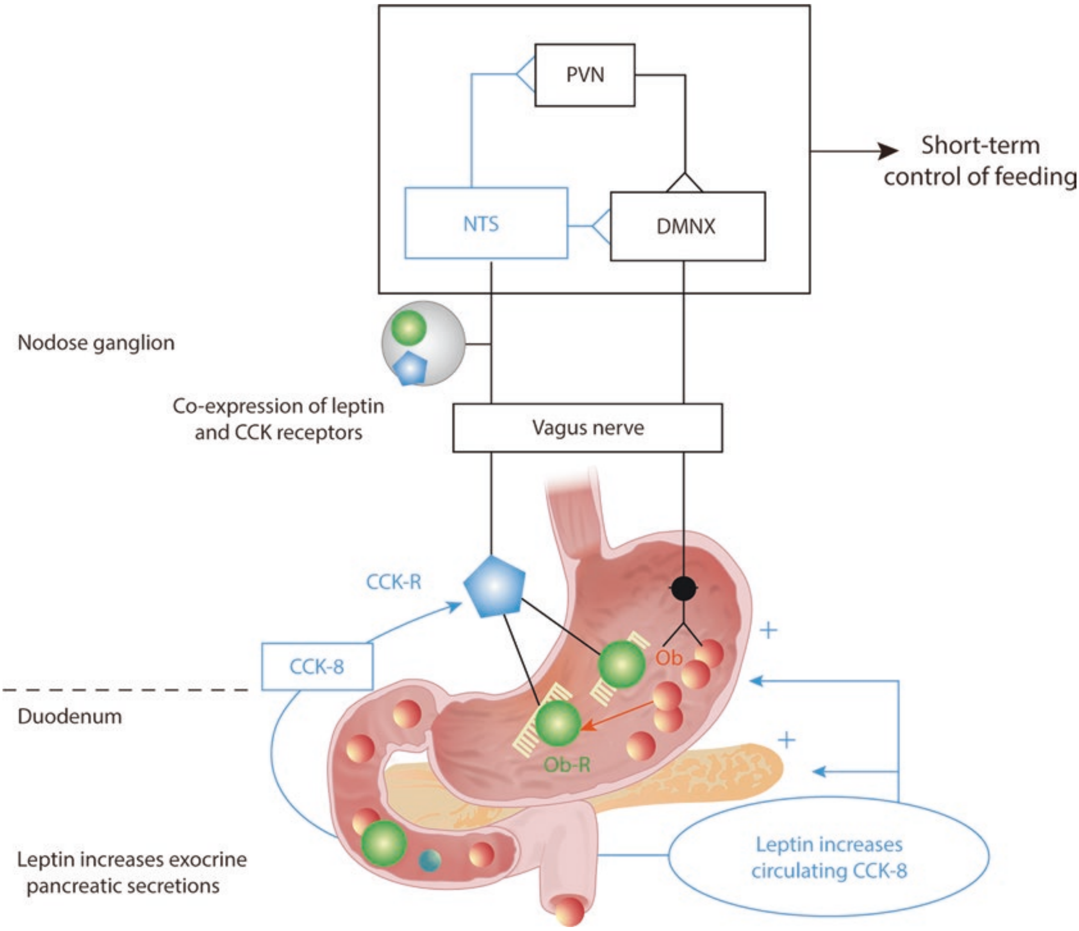
PVN
NTS
DMNX
Short-term control of feeding
Nodose ganglion
Co-expression of leptin and CCK receptors
Vagus nerve
CCK-R
CCK-8
Duodenum
Ob
Ob-R
+
+
Leptin increases exocrine pancreatic secretions
Leptin increases circulating CCK-8

Fig. 4.11 Model of the action of gastric leptin. Leptin (secreted by the stomach) and CCK can be considered as short-term gastrointestinal signals in the control of feeding. These signals locally activate their receptors on vagal terminals to generate signals that are processed in the NTS and the paraventricular nucleus (PVN) of the hypothalamus. The stomach-derived leptin secreted in the lumen enters the intestine in an active form. *CCK-R* cholecystokinin receptor, *DMNX* dorsomotor nucleus of the vagus nerve, *Ob* leptin, *Ob-R* leptin receptors (Modified from Guilmeau et al. [115])

Leptin-secreting endocrine cells are present in the gastric mucosa, but they are few in number and scattered in the gastric mucosa close to blood capillaries [116]. These cells do not reach the gastric lumen but secrete leptin to blood circulation. Since adipocytes are predominant source of leptin, the effects of *H. pylori* infection on the circulating levels of leptin were found to be controvertial (Table 4.4). It has been reported that a rapid increase in the concentration of plasma leptin in response to CCK was involved in the mobilization of a gastric leptin store [129]. Similar to gastric leptin mRNA, higher serum leptin levels were reported in the subjects with *H. pylori* infection compared with *H. pylori*-negative subjects [80, 91, 128]. However, many studies reported no significant difference in plasma leptin according to *H. pylori* infection status [79, 83, 89, 93, 112]. Similarly, the serum leptin level did not change significantly after curing *H. pylori* infection [107, 126]. In summary, *H. pylori* infection seems to enhance gastric leptin production,

Table 4.3 Studies that compared gastric levels of leptin between *H. pylori*-positive and *H. pylori*-negative subjects (including *H. pylori* eradication)

Authors	Subjects	Sex	Age	No.	Specimen	Method	Results in HP (+) subjects
Breidert et al. (1999), USA [112]	Dyspepsia	B	A	39	Antrum and body	Commercial ELISA	↑ Leptin mRNA in HP (+) in only the body
Azuma et al. (2001), Japan [126]	CG	B	A	201 HP (+)	Fundus	RT-PCR	↑ Leptin mRNA in HP (+)/↓ Leptin mRNA after cure (12 weeks)
Salles et al. (2006), France [83]	Hospitalized in geriatric	B	A (>75 years)	62	Body	RT-PCR	↓ Leptin mRNA more so as atrophy increases
Jun et al. (2007), Korea [89]	CG	B	A	63	Body	RT-PCR	↑ Leptin mRNA in HP (+)
Roper et al. (2008), USA [91]	Healthy	M	A	256	Antrum and fundus	Commercial ELISA	→ Leptin in the mucosa
Romo-González, et al. (2017), Mexico City [127]	Healthy	B	C	172	Serum	Commercial ELISA	↓ Leptin in the serum

Articles written in English and assay method were stated

↑ increased (or higher), ↓ decreased (or lower), → no significant change (or difference)

A adults, *B* both, *C* child, *W* women, *M* men, *HP H. pylori*, *No* sample size, *CG* chronic gastritis, *Sick* patients who visited clinics, but the specific condition is not stated, *ELISA* enzyme-linked immunosorbent assay, *RT-PCR* real-time polymerase chain reaction

Table 4.4 Studies that compared the circulating levels of leptin between *H. pylori*-positive and *H. pylori*-negative subjects (including *H. pylori* eradication)

Authors	Subjects	Sex	Age	No.	Specimen	Method	Results after eradication
Breidert et al. (1999), USA [112]	Dyspepsia	B	A	39	Plasma	Commercial ELISA	→
Azuma et al. (2001), Japan [126]	CG	B	A	201 HP (+)	Serum	Commercial ELISA	→ after cure (12 weeks)
Nwokolo et al. (2003), UK [107]	Healthy	B	A	10	Plasma	Commercial RIA	→ after cure (6 weeks)
LanKarani et al. (2004), Iran [128]	Dyspepsia + healthy	B	A	66	Serum	Commercial ELISA	↑
Shiotani et al. (2005), Japan [79]	Healthy	B	A	132	Serum	Commercial ELISA	→
Konturek et al. (2006), Poland [80]	Healthy	B	B	180	Serum	Human RIA	↑
Plonka et al. (2006), Poland [82]	Healthy	B	C	287	Serum	Commercial RIA	↓
Salles et al. (2006), France [83]	Hospitalized in geriatric	B	A (>75 years)	62	Plasma	Commercial RIA	→
Jun et al. (2007), Korea [89]	CG	B	A	63	Plasma	Commercial RIA	→
Roper et al. (2008), USA [91]	Healthy	M	A	256	Serum	Commercial ELISA	↑
Pacifico et al. (2008), Italy [90]	Healthy + sick (GI symptom)	B	C	85	Serum	Commercial RIA	↓ in HP (+) /↑ after cure
Chuang et al. (2009), Taiwan [93][a]	PU + FD	M	A	145	Plasma	Commercial RIA	→
Chuang et al. (2009), Taiwan [93][a]	PU + FD	W	A	196	Plasma	Commercial RIA	→

↑ increased (or higher), ↓ decreased (or lower), → no significant change (or difference)
A adults, *B* both, *C* child, *W* women, *M* men, *HP H. pylori*, *No* sample size, *CG* chronic gastritis, *FD* functional dyspepsia, *GI* gastrointestinal, *PU* peptic ulcer, *Sick* patients who visited clinics, but the specific condition is not stated, *EIA* enzyme immunoassay, *ELISA* enzyme-linked immunosorbent assay, *RIA* radioimmunoassay

[a] Articles written in English and assay method were stated

but its effect on the circulating level is not much. Furthermore, the effect of *H. pylori* eradication on leptin is not conclusive, yet.

4.5 Conclusions

Understanding the changes of acid secretion related to *H. pylori* infection gave basic insights regarding why the same *H. pylori* causes different outcomes among gastric ulcer, DU, GC, or asymptomatic histologic gastritis. Gut hormones such as ghrelin and leptin, which are regulated by the neuronal, hormonal, and immune process under *H. pylori* infection, are involved not only in GI physiology but in systemic energy regulation including adiposity, appetite, or circulation. The change of physiology of G and D cells during *H. pylori* infection and after its eradication has been relatively well evaluated, while those of ghrelin and leptin are still under debate. However, *H. pylori* eradication looks like to upregulate pre-proghrelin mRNA expression and increase the plasma acyl ghrelin level, contributing to the abatement of PDS symptoms [97]. Investigating these hormonal dynamics altered by *H. pylori* infection could provide an important clinical implication in the prevention and treatment of illnesses including obesity, FD, and GI cancer and give clear evidence of eradicating this microorganism.

References

1. Franceschi F, Annalisa T, Teresa DR, Giovanna D, Ianiro G, Franco S, et al. Role of *Helicobacter pylori* infection on nutrition and metabolism. World J Gastroentrol. 2014;20:12809–17.
2. Sachs G, Shin JM, Munson K, Scott DR. Gastric acid-dependent diseases: a twentieth-century revolution. Dig Dis Sci. 2014;59:1358–69.
3. Smolka AJ, Backert S. How *Helicobacter pylori* infection controls gastric acid secretion. J Gastroenterol. 2012;47:609–18.
4. Itoh Z, Takeuchi S, Aizawa I, Honda R. The negative feedback mechanism of gastric acid secretion: significance of acid in the gastric juice in man and dog. Surgery. 1975;77:648–60.
5. Forte JG, Hanzel DK, Urushidani T, Wolosin JM. Pumps and path-ways for gastric HCl secretion. Ann N Y Acad Sci. 1989;574:145–58.
6. Shin JM, Kim N. Pharmacokinetics and pharmacodynamics of the proton pump inhibitors. J Neurogastroenterol Motil. 2013;19:25–35.
7. Spicer Z, Miller ML, Andringa A, Riddle TM, Duffy JJ, Doetschman T, et al. Stomachs of mice lacking the gastric H, K-ATPase -subunit have achlorhydria, abnormal parietal cells, and ciliated metaplasia. J Biol Chem. 2000;275:21555–65.
8. Asano S, Kawada H, Kimura T, Grishin AV, Caplan MJ, Takeguchi N. The roles of carbohydrate chains of the β-subunit on the functional expression of gastric H+, K+-ATPase. J Biol Chem. 2000;275:8324–30.
9. Valle JD. Peptic ulcer disease and related disorders. In: Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL, editors. Harrison's principles of internal medicine. 16th ed. New York: McGraw-Hill; 2005. p. 1746–62.
10. Forte JG, Zhu L. Apical recycling of the gastric parietal cell H, K-ATPase. Annu Rev Physiol. 2010;72:273–96.
11. Black JA, Forte TM, Forte JG. Structure of oxyntic cell membranes during conditions of rest and secretion of HCl as revealed by freeze-fracture. Anat Rec. 1980;196:163–72.
12. Smolka A, Helander HF, Sachs G. Monoclonal antibodies against gastric H^+K^+ ATPase. Am J Physiol. 1983;245:G589–96.
13. Soroka CJ, Chew CS, Hanzel DK, Smolka A, Modlin IM, Goldenring JR. Characterization of membrane and cytoskeletal compartments in cultured parietal cells: immunofluorescence and confocal microscopic examination. Eur J Cell Biol. 1993;60:76–87.
14. Zaki M, Coudron PE, McCuen RW, Harrington L, Chu S, Schubert ML. *H. pylori* acutely inhibits gastric secretion by activating CGRP sensory neurons coupled to stimulation of somatostatin and inhibition of histamine secretion. Am J Physiol Gastrointest Liver Physiol. 2013;304:G715–22.
15. Beales I, Calam J. Interleukin 1β and tumour necrosis factor α inhibit acid secretion in cultured rabbit parietal cells by multiple pathways. Gut. 1998;42:227–34.
16. Prinz C, Neumayer N, Mahr S, Classen M, Schepp W. Functional impairment of rat enterochromaffin-like cells by interleukin 1 beta. Gastroenterology. 1997;112:364–75.
17. Kuipers EJ, Uyterlinde AM, Peña AS, Roosendaal R, Pals G, Nelis GF, et al. Long-term sequelae of *Helicobacter pylori* gastritis. Lancet. 1995;345:1525–8.
18. Takashima M, Furuta T, Hanai H, Sugimura H, Kaneko E. Effects of *Helicobacter pylori* infection on gastric acid secretion and serum gastrin levels in Mongolian gerbils. Gut. 2001;48:765–73.
19. Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ. Attempt to fulfil Koch's postulates for pyloric *Campylobacter*. Med J Aust. 1985;142:436–9.
20. Morris A, Nicholson G. Ingestion of *Campylobacter pyloridis* causes gastritis and raised fasting gastric pH. Am J Gastroenterol. 1987;82:192–9.

21. Graham DY, Alpert LC, Smith JL, Yoshimura HH. Iatrogenic *Campylobacter pylori* infection is a cause of epidemic achlorhydria. Am J Gastroenterol. 1988;83:974–80.
22. Sobala GM, Crabtree JE, Dixon MF, Schorah CJ, Taylor JD, Rathbone BJ, et al. Acute *Helicobacter pylori* infection: clinical features, local and systemic immune response, gastric mucosal histology, and gastric juice ascorbic acid concentrations. Gut. 1991;32:1415–8.
23. Ramsey EJ, Carey KV, Peterson WL, Jackson JJ, Murphy FK, Read NW, et al. Epidemic gastritis with hypochlorhydria. Gastroenterology. 1979;76:1449–57.
24. El-Omar EM, Oien K, El Nujumi A, Gillen D, Wirz A, Dahill S, et al. *Helicobacter pylori* infection and chronic gastric acid hyposecretion. Gastroenterology. 1997;113:15–24.
25. Harford WV, Barnett C, Lee E, Perez-Perez G, Blaser MJ, Peterson WL. Acute gastritis with hypochlorhydria: report of 35 cases with long term follow up. Gut. 2000;47:467–72.
26. Hoffman JS, King WW, Fox JG, Janik D, Cave DR. Rabbit and ferret parietal cell inhibition by *Helicobacter* species. Dig Dis Sci. 1995;40:147–52.
27. Cave D, Vargas M. Effect of a *Campylobacter pylori* protein on acid secretion by parietal cells. Lancet. 1989;2:187–9.
28. Tagkalidis PP, Royce SG, Macrae FA, Bhathal PS. Selective colonization by *Helicobacter pylori* of the deep gastric glands and intracellular canaliculi of parietal cells in the setting of chronic proton pump inhibitor use. Eur J Gastroenterol Hepatol. 2002;14:453–6.
29. Chen XG, Correa P, Offerhaus J, Rodriguez E, Janney F, Hoffmann E, et al. Ultrastructure of the gastric mucosa harboring *Campylobacter*-like organisms. Am J Clin Pathol. 1986;86:575–82.
30. Jablonowski H, Hengels KJ, Krämer N, Geis G, Opferkuch W, Strohmeyer G. Effect of *Helicobacter pylori* on dbc-AMP stimulated acid secretion by human parietal cells. Hepatogastroenterology. 1994;41:546–8.
31. Jablonowski H, Hengels KJ, Kraemer N, Geis G, Opferkuch W, Strohmeyer G. Effects of *Helicobacter pylori* on histamine and carbachol stimulated acid secretion by human parietal cells. Gut. 1994;35:755–7.
32. Furuta T, Baba S, Takashima M, Shirai N, Xiao F, Futami H, et al. H^+/K^+-adenosine triphosphatase mRNA in gastric fundic gland mucosa in patients infected with *Helicobacter pylori*. Scand J Gastroenterol. 1999;34:384–90.
33. Wolfe MM, Nompleggi DJ. Cytokine inhibition of gastric acid secretion—a little goes a long way. Gastroenterology. 1992;102:2177–8.
34. Wallace JL, Cucala M, Mugridge K, Parente L. Secretagogue-specific effects of interleukin-1 on gastric acid secretion. Am J Physiol. 1991;261(4 Pt 1):G559–64.
35. Amedei A, Munarp F, Bella CD, Niccolai E, Benagiano M, Bencini L, et al. *Helicobacter pylori* HP0175 promotes the production of IL-23, IL-6, IL-1β and TGF-β. Eur J Inflamm. 2013;11:261–8.
36. Peek RM Jr, Crabtree JE. *Helicobacter* infection and gastric neoplasia. J Pathol. 2006;208:233–48.
37. Kaneko H, Konagaya T, Kusugami K. *Helicobacter pylori* and gut hormones. J Gastroenterol. 2002;37:77–86.
38. Levi S, Beardshall K, Haddad G, Playford R, Ghosh P, Calam J. *Campylobacter pylori* and duodenal ulcers: the gastrin link. Lancet. 1989;1:1167–8.
39. Beardshall K, Moss S, Gill J, Levi S, Ghosh P, Playford RJ, et al. Suppression of *Helicobacter pylori* reduces gastrin releasing peptide stimulated gastrin release in duodenal ulcer patients. Gut. 1992;33:601–3.
40. Penman E, Wass JA, Butler MG, Penny ES, Price J, Wu P, et al. Distribution and characterisation of immunoreactive somatostatin in human gastrointestinal tract. Regul Pept. 1983;7:53–65.
41. Walsh JH, Dockray GJ, Mitty RD. Gut peptides: biochemistry and physiology. Endocrinologist. 1994;4:487.
42. Kaneko H, Nakada K, Mitsuma T, Uchida K, Furusawa A, Maeda Y, et al. *Helicobacter pylori* infection induces a decrease in immunoreactive-somatostatin concentrations of human stomach. Dig Dis Sci. 1992;37:409–16.
43. Zavros Y, Paterson A, Lambert J, Shulkes A. Expression of progastrin-derived peptides and somatostatin in fundus and antrum of nonulcer dyspepsia subjects with and without *Helicobacter pylori* infection. Dig Dis Sci. 2000;45:2058–64.
44. Tzaneva M, Julianov A. Chromogranin A-, somatostatin-and serotonin-containing endocrine cells in the corporal gastric mucosa of patients with *Helicobacter pylori* associated chronic gastritis. Endocr Regul. 1999;33:79–82.
45. Moss SF, Legon S, Bishop AE, Polak JM, Calam J. Effect of *Helicobacter pylori* on gastric somatostatin in duodenal ulcer disease. Lancet. 1992;340:930–2.
46. Osawa H, Kita H, Ohnishi H, Hoshino H, Mutoh H, Ishino Y, et al. *Helicobacter pylori* eradication induces marked increase in H^+/K^+-adenosine triphosphatase expression without altering parietal cell number in human gastric mucosa. Gut. 2006;55:152–7.
47. El-Omar EM, Oien K, Murray LS, El-Nujumi A, Wirz A, Gillen D, et al. Increased prevalence of precancerous change in relatives of gastric cancer patients: critical role of *H. pylori*. Gastroenterology. 2000;118:22–30.
48. Valle J, Kekki M, Sipponen P, Ihamäki T, Siurala M. Long-term course and consequences of *Helicobacter pylori* gastritis. Results of a 32-year follow-up study. Scand J Gastroenterol. 1996;31:546–50.

49. Iijima K, Sekine H, Koike T, Imatani A, Ohara S, Shimosegawa T. Long-term effect of *Helicobacter pylori* eradication on the reversibility of acid secretion in profound hypochlorhydria. Aliment Pharmacol Ther. 2004;19:1181–8.
50. Kamada T, Haruma K, Inoue K, Manabe N, Matsumoto H, Kusunoki H, et al. Time trends in *Helicobacter pylori* infection and trophic gastritis over 40 years in Japan. Helicobacter. 2014;20:192–8.
51. Hwang YJ, Kim N, Lee HS, Lee JB, Choi YJ, Yoon H, et al. Reversibility of atrophic gastritis and intestinal metaplasia after *Helicobacter pylori* eradication—a prospective study for up to 10 years. Aliment Pharmacol Ther. 2018;47:380–90.
52. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 1999;402:656–60.
53. Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, et al. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab. 2001;86:4753–8.
54. Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, et al. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology. 2000;141:4255–61.
55. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. Endocrinology. 2000;141:4797–800.
56. Korbonits M, Bustin SA, Kojima M, Jordan S, Adams EF, Lowe DG, et al. The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumors. J Clin Endocrinol Metab. 2001;86:881–7.
57. Papotti M, Ghè C, Cassoni P, Catapano F, Deghenghi R, Ghigo E, et al. Growth hormone secretagogue binding sites in peripheral human tissues. J Clin Endocrinol Metab. 2000;85:3803–7.
58. Hosoda H, Kojima M, Matsuo H, Kangawa K. Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. Biochem Biophysic Res Commun. 2000;279:909–13.
59. Nishi Y, Yoh J, Hiejima H, Kojima M. Structures and molecular forms of the ghrelin-family peptides. Peptides. 2011;32:2175–82.
60. Gutierrez JA, Solenberg PJ, Perkins DR, Willency JA, Knierman MD, Jin Z, et al. Ghrelin octanoylation mediated by an orphan lipid transferase. Proc Natl Acad Sci U S A. 2008;105:6320–5.
61. Kang K, Schmahl J, Lee JM, Garcia K, Patil K, Chen A, et al. Mouse ghrelin-O-acyltransferase (GOAT) plays a critical role in bile acid reabsorption. FASEB J. 2012;26:259–71.
62. Stengel A, Keire D, Goebel M, Evilevitch L, Wiggins B, Taché Y, et al. The RAPID method for blood processing yields new insight in plasma concentrations and molecular forms of circulating gut peptides. Endocrinology. 2009;150:5113–8.
63. Inui A, Asakawa A, Bowers CY, Mantovani G, Laviano A, Meguid MM, et al. Ghrelin, appetite, and gastric motility: the emerging role of the stomach as an endocrine organ. FASEB J. 2004;18:439–56.
64. Tack J, Depoortere I, Bisschops R, Verbeke K, Janssens J, Peeters T. Influence of ghrelin on gastric emptying and meal-related symptoms in idiopathic gastroparesis. Aliment Pharmacol Ther. 2005;22:847–53.
65. Murray CD, Martin NM, Patterson M, Taylor SA, Ghatei MA, Kamm MA, et al. Ghrelin enhances gastric emptying in diabetic gastroparesis: a double blind, placebo controlled, crossover study. Gut. 2005;54:1693–8.
66. Fujimiya M, Asakawa A, Ataka K, Kato I, Inui A. Different effects of ghrelin, des-acyl ghrelin and obestatin on gastroduodenal motility in conscious rats. World J Gastroenterol. 2008;14:6318–26.
67. Fujimiya M, Asakawa A, Ataka K, Chen CY, Kato I, Inui A. Ghrelin, des-acyl ghrelin, and obestatin: regulatory roles on the gastrointestinal motility. Int J Pept. 2010;2010:305192.
68. Akamizu T, Takaya K, Irako T, Hosoda H, Teramukai S, Matsuyama A, et al. Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. Eur J Endocrinol. 2004;150:447–55.
69. Sánchez J, Oliver P, Palou A, Picó C. The inhibition of gastric ghrelin production by food intake in rats is dependent on the type of macronutrient. Endocrinology. 2004;145:5049–55.
70. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature. 2000;407:908–13.
71. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. Nature. 2001;409:194–8.
72. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, et al. Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab. 2001;86:5992–5.
73. Peeters TL. Central and peripheral mechanisms by which ghrelin regulates gut motility. J Physiol Pharmacol. 2003;54 (Suppl 4):95–103.
74. Isomoto H, Ueno H, Saenko VA, Mondal MS, Nishi Y, Kawano N, et al. Impact of *Helicobacter* pylori infection on gastric and plasma ghrelin dynamics in humans. Am J Gastroenterol. 2005;100:1711–20.
75. Gokcel A, Gumurdulu Y, Kayaselcuk F, Serin E, Ozer B, Ozsahin AK, et al. *Helicobacter pylori* has no effect on plasma ghrelin levels. Eur J Endocrinol. 2003;148:423–6.
76. Isomoto H, Nakazato M, Ueno H, Date Y, Nishi Y, Mukae H, et al. Low plasma ghrelin levels in patients

with *Helicobacter pylori*–associated gastritis. Am J Med. 2004;117:429–32.

77. Isomoto H, Ueno H, Nishi Y, Wen CY, Nakazato M, Kohno S. Impact of *Helicobacter pylori* infection on ghrelin and various neuroendocrine hormones in plasma. World J Gastroenterol. 2005;11:1644–8.
78. Osawa H, Nakazato M, Date Y, Kita H, Ohnishi H, Ueno H, et al. Impaired production of gastric ghrelin in chronic gastritis associated with *Helicobacter pylori*. J Clin Endocrinol Metab. 2005;90:10–6.
79. Shiotani A, Miyanishi T, Uedo N, Iishi H. *Helicobacter pylori* infection is associated with reduced circulating ghrelin levels independent of body mass index. Helicobacter. 2005;10:373–8.
80. Konturek PC, Cześnikiewicz-Guzik M, Bielanski W, Konturek SJ. Involvement of *Helicobacter pylori* infection in neuro-hormonal control of food intake. J Physiol Pharmacol. 2006;57:67–81.
81. Plonka M, Konturek PC, Bielanski W, Pawlik T, Brzozowski T, Konturek SJ. Relationship between ghrelin and *Helicobacter pylori* infection in Polish adult shepherds and their children. Aliment Pharmacol Ther. 2006;24 (Suppl 4):160–8.
82. Plonka M, Bielanski W, Konturek SJ, Targosz A, Sliwowski Z, Dobrzanska M, et al. *Helicobacter pylori* infection and serum gastrin, ghrelin and leptin in children of Polish shepherds. Dig Liver Dis. 2006;38:91–7.
83. Salles N, Ménard A, Georges A, Salzmann M, de Ledinghen V, de Mascarel A, et al. Effects of *Helicobacter pylori* infection on gut appetite peptide (leptin, ghrelin) expression in elderly inpatients. J Gerontol A Biol Sci Med Sci. 2006;61:1144–50.
84. Alonso N, Granada ML, Salinas I, Reverter JL, Flores L, Ojanguren I, et al. Plasma ghrelin concentrations in type 1 diabetic patients with autoimmune atrophic gastritis. Eur J Endocrinol. 2007;157:763–9.
85. An JY, Choi MG, Noh JH, Sohn TS, Jin DK, Kim S. Clinical significance of ghrelin concentration of plasma and tumor tissue in patients with gastric cancer. J Surg Res. 2007;143:344–9.
86. Cindoruk M, Yetkin I, Deger SM, Karakan T, Kan E, Unal S. Influence of *H. pylori* on plasma ghrelin in patients without atrophic gastritis. World J Gastroenterol. 2007;13:1595–8.
87. D'Onghia V, Leoncini R, Carli R, Santoro A, Giglioni S, Sorbellini F, et al. Circulating gastrin and ghrelin levels in patients with colorectal cancer: correlation with tumour stage, *Helicobacter pylori* infection and BMI. Biomed Pharmacother. 2007;61:137–41.
88. de Martel C, Haggerty TD, Corley DA, Vogelman JH, Orentreich N, Parsonnet J. Serum ghrelin levels and risk of subsequent adenocarcinoma of the esophagus. Am J Gastroenterol. 2007;102:1166–72.
89. Jun DW, Lee OY, Lee YY, Choi HS, Kim TH, Yoon BC. Correlation between gastrointestinal symptoms and gastric leptin and ghrelin expression in patients with gastritis. Dig Dis Sci. 2007;52:2866–72.
90. Pacifico L, Anania C, Osborn JF, Ferrara E, Schiavo E, Bonamico M, et al. Long-term effects of *Helicobacter pylori* eradication on circulating ghrelin and leptin concentrations and body composition in prepubertal children. Eur J Endocrinol. 2008;158:323–32.
91. Roper J, Francois F, Shue PL, Mourad MS, Pei Z, Olivares de Perez AZ, et al. Leptin and ghrelin in relation to *Helicobacter pylori* status in adult males. J Clin Endocrinol Metab. 2008;93:2350–7.
92. Shak JR, Roper J, Perez-Perez GI, Tseng C, Francois F, Gamagaris Z, et al. The effect of laparoscopic gastric banding surgery on plasma levels of appetite-control, insulinotropic, and digestive hormones. Obes Surg. 2008;18:1089–96.
93. Chuang CH, Sheu BS, Yang HB, Lee SC, Kao AW, Cheng HC, et al. Gender difference of circulating ghrelin and leptin concentrations in chronic *Helicobacter pylori* infection. Helicobacter. 2009;14:54–60.
94. Gao XY, Kuang HY, Liu XM, Duan P, Yang Y, Ma ZB. Circulating ghrelin/obestatin ratio in subjects with *Helicobacter pylori* infection. Nutrition. 2009;25:506–11.
95. Kawashima J, Ohno S, Sakurada T, Takabayashi H, Kudo M, Ro S, et al. Circulating acylated ghrelin level decreases in accordance with the extent of atrophic gastritis. J Gastroenterol. 2009;44:1046–54.
96. Campana D, Nori F, Pagotto U, De Iasio R, Morselli-Labate AM, Pasquali R, et al. Plasma acylated ghrelin levels are higher in patients with chronic atrophic gastritis. Clin Endocrinol. 2007;67:761–6.
97. Choi YJ, Kim N, Yoon H, Shin CM, Park YS, Park JH, et al. Increase in plasma acyl ghrelin is associated with amelioration of dyspepsia following *Helicobacter pylori* eradication. J Gastroenterol. 2016;51:548–59.
98. Nweneka CV, Prentice AM. *Helicobacter pylori* infection and circulating ghrelin levels—a systematic review. BMC Gastroenterol. 2011;11:7.
99. Isomoto H, Nishi Y, Ohnita K, Mizuta Y, Kohno S, Ueno H, et al. The relationship between plasma and gastric ghrelin levels and strain diversity in *Helicobacter pylori* virulence. Am J Gastroenterol. 2005;100:1425–7.
100. Liew PL, Lee WJ, Lee YC, Chen WY. Gastric ghrelin expression associated with *Helicobacter pylori* infection and chronic gastritis in obese patients. Obes Surg. 2006;16:612–9.
101. Checchi S, Montanaro A, Pasqui L, Ciuoli C, Cevenini G, Sestini F, et al. Serum ghrelin as a marker of atrophic body gastritis in patients with parietal cell antibodies. J Clin Endocrinol Metab. 2007;92:4346–51.
102. Lee ES, Yoon YS, Park CY, Kim HS, Um TH, Baik HW, et al. Eradication of *Helicobacter pylori* increases ghrelin mRNA expression in the gastric mucosa. J Korean Med Sci. 2010;25:265–71.
103. Jang EJ, Park SW, Park JS, Park SJ, Hahm KB, Paik SY, et al. The influence of the eradication of *Helicobacter pylori* on gastric ghrelin, appetite, and body mass index in patients with peptic ulcer

disease. J Gastroenterol Hepatol. 2008;23 (Suppl 2):S278–85.

104. Osawa H, Kita H, Ohnishi H, Nakazato M, Date Y, Bowlus CL, et al. Changes in plasma ghrelin levels, gastric ghrelin production, and body weight after *Helicobacter pylori* cure. J Gastroenterol. 2006;41:954–61.
105. Tatsuguchi A, Miyake K, Gudis K, Futagami S, Tsukui T, Wada K, et al. Effect of *Helicobacter pylori* infection on ghrelin expression in human gastric mucosa. Am J Gastroenterol. 2004;99:2121–7.
106. Choe YH, Lee JH, Lee HJ, Paik KH, Jin DK, Song SY, et al. Ghrelin levels in gastric mucosa before and after eradication of *Helicobacter pylori*. Gut Liver. 2007;1:132–7.
107. Nwokolo CU, Freshwater DA, O'Hare P, Randeva HS. Plasma ghrelin following cure of *Helicobacter pylori*. Gut. 2003;52:637–40.
108. Czesnikiewicz-Guzik M, Loster B, Bielanski W, Guzik TJ, Konturek PC, Zapala J, et al. Implications of oral *Helicobacter pylori* for the outcome of its gastric eradication therapy. J Clin Gastroenterol. 2007;41:145–51.
109. Lee KJ, Cha DY, Cheon SJ, Yeo M, Cho SW. Plasma ghrelin levels and their relationship with gastric emptying in patients with dysmotility-like functional dyspepsia. Digestion. 2009;80:58–63.
110. Takamori K, Mizuta Y, Takeshima F, Akazawa Y, Isomoto H, Ohnita K, et al. Relation among plasma ghrelin level, gastric emptying, and psychologic condition in patients with functional dyspepsia. J Clin Gastroenterol. 2007;41:477–83.
111. Shindo T, Futagami S, Hiratsuka T, Horie A, Hamamoto T, Ueki N, et al. Comparison of gastric emptying and plasma ghrelin levels in patients with functional dyspepsia and non-erosive reflux disease. Digestion. 2009;79:65–72.
112. Breidert M, Miehlke S, Glasow A, Orban Z, Stolte M, Ehninger G, et al. Leptin and its receptor in normal human gastric mucosa and in *Helicobacter pylori*-associated gastritis. Scand J Gastroenterol. 1999;34:954–61.
113. Sobhani I, Bado A, Vissuzaine C, Buyse M, Kermorgant S, Laigneau JP, et al. Leptin secretion and leptin receptor in the human stomach. Gut. 2000;47:178–83.
114. Cammisotto P, Bendayan M. A review on gastric leptin: the exocrine secretion of a gastric hormone. Anat Cell Biol. 2012;45:1–16.
115. Guilmeau S, Buyse M, Bado A. Gastric leptin: a new manager of gastrointestinal function. Curr Opin Pharmacol. 2004;4:561–6.
116. Zhao Z, Sakata I, Okubo Y, Koike K, Kangawa K, Sakai T. Gastric leptin, but not estrogen and somatostatin, contributes to the elevation of ghrelin mRNA expression level in fasted rats. J Endocrinol. 2008;196:529–38.
117. Moss SF, Calam J. Acid secretion and sensitivity to gastrin in patients with duodenal ulcer: effect of eradication of *Helicobacter pylori*. Gut. 1993;34:888–92.
118. Moran TH, McHugh PR. Cholecystokinin suppresses food intake by inhibiting gastric emptying. Am J Physiol. 1982;242:R491–7.
119. Wang L, Barachina MD, Martínez V, Wei JY, Taché Y. Synergistic interaction between CCK and leptin to regulate food intake. Regul Pept. 2000;92:79–85.
120. Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Ørskov C, Ritzel R, et al. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am J Physiol. 1997;273(5 Pt 1):E981–8.
121. Naveilhan P, Hassani H, Canals JM, Ekstrand AJ, Larefalk A, Chhajlani V, et al. Normal feeding behavior, body weight and leptin response require the neuropeptide Y Y2 receptor. Nat Med. 1999;5:1188–93.
122. Andrews PL, Sanger GJ. Abdominal vagal afferent neurones: an important target for the treatment of gastrointestinal dysfunction. Curr Opin Pharmacol. 2002;2:650–6.
123. Burdyga G, Spiller D, Morris R, Lal S, Thompson DG, Saeed S, et al. Expression of the leptin receptor in rat and human nodose ganglion neurones. Neuroscience. 2002;109:339–47.
124. Cammisotto PG, Bendayan M. Leptin secretion by with adipose tissue and gastric mucosa. Histol Histopathol. 2007;22:199–210.
125. Weller A, Smith GP, Gibbs J. Endogenous cholecystokinin reduces feeding in young rats. Science. 1990;247:1589–91.
126. Azuma T, Suto H, Ito Y, Ohtani M, Dojo M, Kuriyama M, et al. Gastric leptin and *Helicobacter pylori* infection. Gut. 2001;49:324–9.
127. Romo-González C, Mendoza E, Mera RM, Coria-Jiménez R, Chico-Aldama P, Gomez-Diaz R, et al. *Helicobacter pylori* infection and serum leptin, obestatin, and ghrelin levels in Mexican schoolchildren. Pediatr Res. 2017;82:607–13.
128. Lankarani KB, Moghadami M, Masoumpoor M, Geramizadeh B, Omrani GR. Serum leptin level in patients with functional dyspepsia. Dig Liver Dis. 2004;36:717–21.
129. Bado A, Levasseur S, Attoub S, Kermorgant S, Laigneau JP, Bortoluzzi MN, et al. The stomach is a source of leptin. Nature. 1998;394:790–3.

H. pylori Virulence Factors: Toxins (CagA, VacA, DupA, OipA, IceA)

5

Jung Mogg Kim

Abstract

Helicobacter pylori (*H. pylori*) specifically colonizes the human stomach. The majority of bacteria live in the mucus layer overlying gastric epithelial cells and only a small proportion of bacteria are found interacting with the epithelial cells. To succeed in long-term colonization, *H. pylori* has developed a unique set of virulence factors, which allow survival in a harsh ecological niche. Clinical outcomes associated with *H. pylori* infections are largely mediated by a complex interaction between bacterial, host, and environmental factors. Over the past year, a variety of studies focusing on both host and bacterial factors have proceeded. Among the bacterial factors that contribute to the pathophysiology associated with *H. pylori* infections, it is remarkable that the cytotoxin-associated gene A (CagA) protein is delivered into gastric epithelial cells via bacterial type IV secretion system, leading to the induction of diverse immune responses and gastroduodenal diseases. The vacuolating cytotoxin (VacA) is also one of the most important virulence factors and functions as an intracellular-acting protein exotoxin, in which one of the most important target sites for VacA is the mitochondrial inner membrane in the host. In addition, the outer membrane inflammatory protein (OipA), induced by contact with epithelium (IceA), and duodenal ulcer promoting (*dupA*) gene have emerged as virulence factors, although the specific genes involved in virulence are still being determined. This chapter summarizes the results of the most relevant studies regarding *H. pylori* virulence factors, such as CagA, VacA, OipA, IceA, and *dupA* genes, and discusses their molecular mechanism for generating gastrointestinal diseases.

Keywords

CagA · *Helicobacter pylori* · VacA · Virulence factor

5.1 Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative spiral-shaped bacillus, known to reside in the human stomach. It is presumed that the pathogen has undergone evolution along with human beings, ever since it was introduced in Africa at least 50,000 years ago. Accordingly, the primitive properties of *H. pylori* (e.g., the virulence factor) have changed over evolutionary time, whereby each *H. pylori* strain in different locales may have taken different evolution pathways. These bacteria have pathogenetically been identified as a cause of acute and chronic gastritis, peptic ulcers, and gas-

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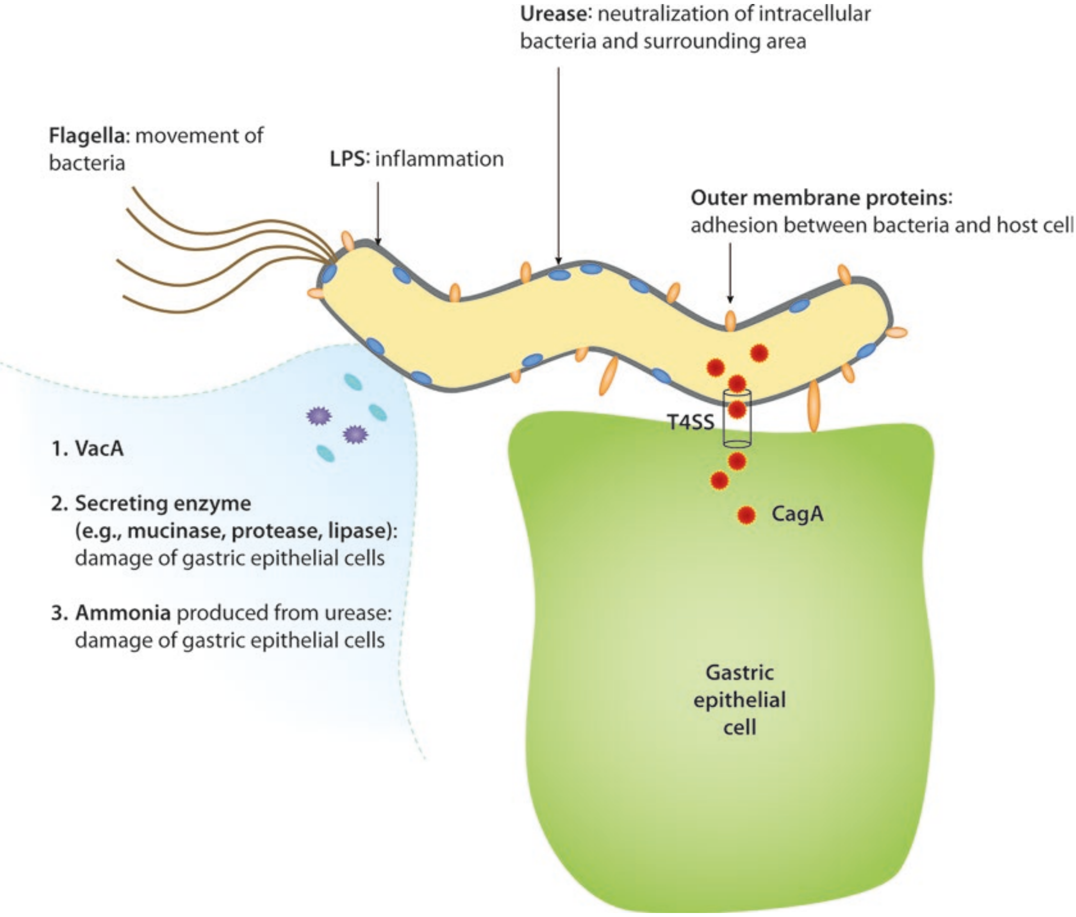
Urease: neutralization of intracellular bacteria and surrounding area
Flagella: movement of bacteria
LPS: inflammation
Outer membrane proteins: adhesion between bacteria and host cell
T4SS
CagA
1. VacA
2. Secreting enzyme (e.g., mucinase, protease, lipase): damage of gastric epithelial cells
3. Ammonia produced from urease: damage of gastric epithelial cells
Gastric epithelial cell

Fig. 5.1 Virulence factors of *H. pylori* and their functions. *CagA* cytotoxin-associated gene A, *T4SS* type IV secretion system, *VacA* vacuolating cytotoxin, *LPS* lipopolysaccharide

tric cancer (GC). By definition, the potential for pathogens to cause disease is referred to as virulence, and a factor associated with this virulence is called the virulence factor. In the case of *H. pylori*, the virulence factors for gastrointestinal (GI) diseases consist of its structural components and secretory materials. Specifically, its structural components include lipopolysaccharide, outer membrane proteins (OMPs), flagella, and the type IV secretion system (T4SS), while its secretory materials comprise ammonia by urease, cytotoxin-associated gene A (CagA), vacuolating cytotoxin (VacA), and other secretory enzymes [1] (Fig. 5.1).

According to geographical differences with person-to-person variation, *H. pylori* infection rate and disease occurrence are known to be different, supposedly due to the various host factors, types of food intake, environmental factors, and so on. In addition, the genetic polymorphism that controls the host inflammatory responses can either increase or decrease the degree of the inflammation and thereby contribute to these different clinical aspects of diseases upon *H. pylori* infection [2]. More on the topic regarding genetic polymorphism will be described in the next chapter, while this chapter focuses on *H. pylori* virulence factors, among which are CagA, VacA, outer inflammatory protein (OipA), induced by contact with the epithelium (IceA), and duodenal ulcer promoting (*dupA*) gene.

5.2 Cytotoxin-Associated Gene A (CagA)

CagA is by far one of the most actively investigated virulence factors of *H. pylori*. *H. pylori* strains isolated from patients can be classified according to the presence or absence of the *cagA* gene, either into the "*cagA*-positive" strain or the "*cagA*-negative" strain. The following experimental results concordantly suggest the possibility of the *cagA* gene being an oncogenic gene of *H. pylori*. Infection of a Mongolian gerbil with a *cagA*-positive strain results in GC, whereas the exact same strain with *cagA* mutated (the isogenic *cagA* mutant strain) has not been observed to cause GC [3]. In addition, transgenic mice into which the *cagA* gene was artificially transformed were reported to develop GC [4]. However, in Western countries, *cagA*-positive strains are reportedly more commonly isolated from gastric ulcer or GC patients than *cagA*-negative strains [5, 6], whereas in East Asian countries including South Korea, noteworthily, most of the isolated strains have the *cagA* gene regardless of the GI diseases [7]. Therefore, rather than the association of specific GI diseases solely with the presence of the *cagA* gene alone, it would be more appropriate to comprehensively take other virulence factors and host genetic susceptibility into account.

5.2.1 cag Pathogenicity Island (cag *PAI*)

H. pylori strains are considered to be a highly heterogeneous pathogenic entity associated with genetic recombination and gene mutability. As a result of lengthy evolutionary periods of time, therefore, genetic variants originating from even a single strain are found in the stomach of one individual [8]. As a result, although this leads to difficulty in identifying the correlation of virulence factors with diseases, a specific genetic region, called the *cag* pathogenicity island (*cag* PAI), has been presumed to be associated with tumor formation. The *cag* PAI is the 40-kb DNA insertion element in the bacterial genes, where about 32 genes—which are used in encoding the genetic information for *cagA*, the type IV secretion system (T4SS), etc.—are located [9]. Therefore, the CagA protein can be considered to be a marker for the presence of the *cag* PAI gene. Specifically, *cag* PAI can be found in 60–70% of the strains isolated in Western countries, whereas it is found in almost every strain isolated in East Asia. The *cagA*-positive strains are observed adhering to gastric epithelial cells or being located nearby, whereas most of the *cagA*-negative strains are identified in mucus layers [10]. Therefore, the *cag* PAI genotype is a probable factor attributable to influencing the colonization location inside the stomach.

T4SS, which has a needle-shaped protrusion, is involved in transporting the materials from the bacterial cytoplasm into host cells. This structure is found not only in *H. pylori* but also in other bacteria such as genera *Bordetella*, *Bartonella*, *Legionella*, and *Anaplasma*. The bacterial cytoplasmic materials that are injected by *H. pylori* T4SS consist only of the peptidoglycan of the cellular wall component and the CagA protein [11]. When inactivating a gene encoding CagE, which is a protein component of *H. pylori* T4SS, the *H. pylori* proteins no longer move into the gastric epithelial cells. CagL, an adhesion factor, binds to the α5β1 integrin of the gastric epithelial cells in order to activate the CagA protein, which, in turn, stimulates the transport of toxic materials into the host cells [12]. In addition, CagL, known to bind to integrin and fibronectin, participates in bridging the bacterial T4SS and the host's α5β1 integrin, which leads to the activation of the focal adhesion kinase (FAK) and Src signaling pathway of the host cells. Meanwhile, CagA, CagI, and CagY bind to the β1 integrin, thus inducing the structural transformation of integrin heterodimer in order to facilitate moving the toxic material [13].

Effects following intracellular delivery of CagA have been proposed as follows [9] (Fig. 5.2): translocation of CagA by the bacterial T4SS may lead to activation of host signaling pathways such as promoting epithelial responses with carcinogenic potential. CagA is phosphorylated by Src and Abl kinases. Consequently, phosphorylated CagA acti-

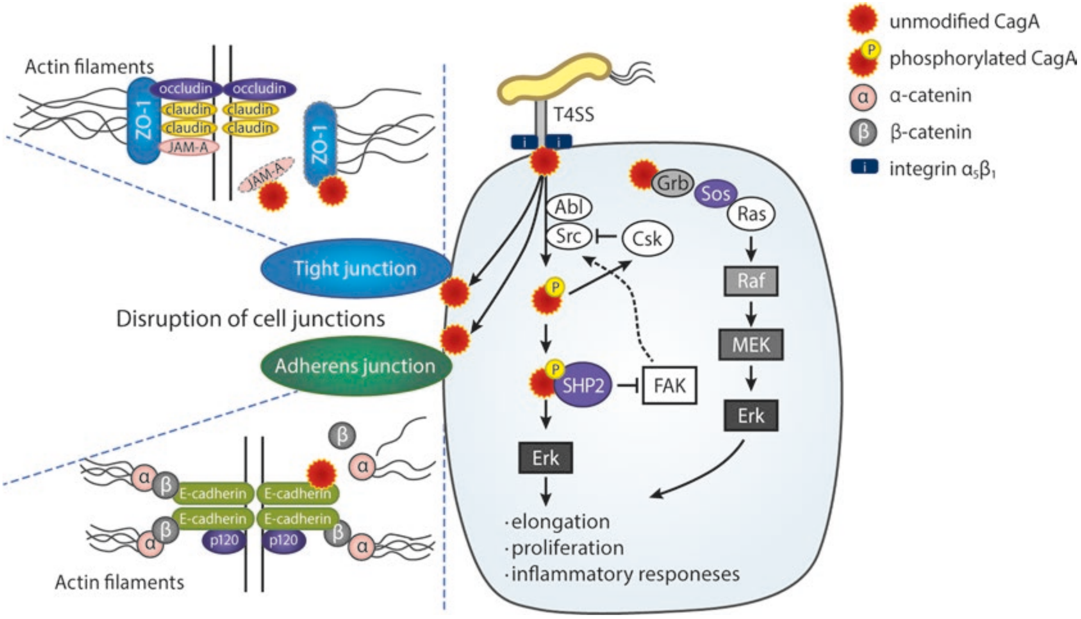
unmodified CagA
P
phosphorylated CagA
α
α-catenin
β
β-catenin
i
integrin α5β1
Actin filaments
ZO-1
occludin
occludin
claudin
claudin
claudin
claudin
JAM-A
JAM-A
ZO-1
Tight junction
Disruption of cell junctions
Adherens junction
β
α
α
β
E-cadherin
E-cadherin
E-cadherin
E-cadherin
β
α
p120
p120
β
α
Actin filaments
T4SS
i
i
Abl
Src
Csk
Grb
Sos
Ras
Raf
MEK
Erk
P
P
SHP2
FAK
Erk
·elongation
·proliferation
·inflammatory responeses

Fig. 5.2 Effects of intracellular delivery of CagA on gastric epithelial cells. Translocation of CagA by the *H. pylori cag* type IV secretion system (T4SS) leads to the activation of host signaling pathways that promote morphologic changes and proliferation of gastric epithelial cells, in which their responses may be associated with carcinogenic potential. Unmodified CagA can also associate with the tight junction proteins ZO-1 and JAM-A as well as the adherens junction protein such as E-cadherin, leading to disrupting the cell junctional complexes. In addition, these signaling are associated with the induction of inflammatory responses. *SHP2* the Src homology-2 domain-containing phosphatase 2, *Csk* Src tyrosine kinase (Modified from Noto and Peek [9])

vates the Src homology-2 domain-containing phosphatase 2 (SHP2) and the Erk. Activated Erk signal is associated with morphological changes of gastric epithelial cells, including cellular elongation. In addition, the interaction between phosphorylated CagA and SHP2 leads to inactivation of FAK, which can activate Src. In this model, excessive phosphorylation of CagA can be inhibited by c-Src kinase (Csk). Therefore, unphosphorylated CagA (unmodified CagA) leads to changes in the epithelial cell motility and proliferation through binding Grb/Sos/Ras and to activation of the Raf/MEK/Erk pathway. On the other hand, unphosphorylated CagA can associate with the tight junction proteins ZO-1 and JAM-A as well as the adherens junction protein E-cadherin, leading to dysregulated junctional complexes [9]. Many studies have been conducted to elucidate molecular mechanisms regarding the CagA-induced clinical outcomes, and it expects to reveal new signaling pathway.

5.2.2 Diversity of the *cagA* Gene

As mentioned, the *cagA* gene is highly heterogeneous among each isolated strain from patients. In particular, the numbers for the repeat sequence located at the 3′ region of the *cagA* gene are different across *H. pylori* strains. The repeat sequence is primarily classified into two types: a first repeat and a second repeat. Specifically, the sequences for the second repeat region in the strains isolated from Eastern and Western show substantial differences [6]. The repeat region of the CagA protein has the Glu-Pro-Ile-Tyr-Ala (EPIYA) motif, whose tyrosine (Tyr) residue, being phosphorylated, will be described next. The first repeat region is named sequentially, the EPIYA-A and EPIYA-B segments, and similarly, the second repeat region contains the EPIYA-C and EPIYA-D segments [14].

5.2.3 The Relevance Between the EPIYA Segment and Pathogenicity of CagA

Theories have been recently established for GCs in relation to the number of EPIYA segments. Compared to strains containing only one EPIYA-C segment, published studies have demonstrated that GC is twice as likely to occur in a patient infected by strains with more than one EPIYA-C segment (e.g., ABC vs. ABCCC) [15, 16]. However, it may be rather questionable to conclude pathogenicity based upon only the number of repeat sequences due to the following considerations: first of all, *H. pylori* strains isolated in Eastern Asia contain mostly only one EPIYA-C segment [17], and, secondly, *H. pylori* strains with multiple EPIYA segments in the repeat region have lower resistance against gastric acids [18], which entails a possible hypothesis that the *H. pylori* strain with multiple EPIYA segments survive upon atrophic gastritis or GCs only in the case of their lower secretion of gastric acids.

5.2.4 Tyrosine Phosphorylation of CagA

Tyrosine residues of the EPIYA motif in CagA protein can be phosphorylated, which then plays a significant role in the pathogenicity of *H. pylori*. Upon injecting CagA into gastric epithelial cells via T4SS, the tyrosine residues of the EPIYA motif undergo phosphorylation by Src or Abl-family tyrosine phosphorylating enzymes [19–21]. Meanwhile, approximately 20 substances in the host cells are estimated to bind to CagA [22], among which about 10 host cell proteins are phosphorylation-dependent binding partners to CagA [22, 23]. The most representative example of this type of interaction with phosphorylated CagA is the SHP2. SHP2 binds with EPIYA-B, EPIYA-C, and EPIYA-D segments [14]. In particular, the fragment sequence of phosphorylated tyrosine in the EPIYA-D segment (EPIYATIDF) perfectly matches with a high-affinity-binding sequence of the SH2 domain of SHP2. In addition, the EPIYA-A and EPIYA-B segments show a tendency to bind with C-terminal Src tyrosine kinase (Csk), whereas the EPIYA-C segment preferably binds with Ras-GAP and Grb7 [23]. In conclusion, phosphorylated CagA, upon interaction with proteins in host cells, may induce morphological changes of gastric epithelial cells and chromosomal instability [11].

5.2.5 Phosphorylation-Independent Signaling of CagA

Unphosphorylated (unmodified) CagA proteins are also able to induce various responses in host cells. More than ten host cell proteins are known to interact with unphosphorylated CagA [22]. Additionally, in the host cell, the CagA proteins can form phosphorylation-independent dimerization, whose binding contact site is estimated to be the CagA multimerization sequence (FPLxRxxxVxDLSKVG) [24]. In other words, the CagA multimerization sequence binds to partitioning-defective 1 (PAR1)/microtubule affinity-regulating kinase (MARK) in order to form a complex, where the unphosphorylated CagA component of this complex inhibits PAR1 activity in order to induce a variety of cellular transformation including the loss of cell polarity [24–26]. The CagA multimerization sequence is also called the “MARK2/PAR1b kinase inhibitor (MKI)” [27]. The same region of CagA, which can be involved in the interaction with the c-Met of host cells, is called the “conserved repeat responsible for phosphorylation-independent activity (CRPIA)” [28]. The interaction between CRPIA and c-Met can induce the activation of transcription factors, β-catenin, and nuclear factor (NF)-κB, which, respectively, can stimulate cell growth and the inflammation response. For reference, nowadays, CagA multimerization, MKI, and CRPIA terminologies are used interchangeably, but there has been an increasing need to unify the terminologies from the perspective of all referring to the same genetic sequence.

The host responses incurred by unphosphorylated CagA are exemplified in the destruction of the cell-cell junction between gastric epithelial cells, loss of cell polarity, induction of inflammatory responses, and stimulation of mitogenic responses. Moreover, the unphosphorylated CagA is involved in the interaction with the runt-related transcription factor 3 (RUNX3), where RUNX3, a tumor suppressor, is observed to be mostly in the inactivated status in GC cells. Therefore, it is postulated that CagA induces ubiquitination of RUNX3 and its decomposition, which, in turn, causes GC [29].

5.3 Vacuolating Cytotoxin (VacA)

VacA can induce cellular vacuolation and cause direct damage to human cells. Since there is a correlation between this virulence factor and the severity of gastric disease in persons infected with *H. pylori*, VacA, in addition to CagA, is possibly another important factor in the pathophysiology of *H. pylori*.

5.3.1 VacA Structure

VacA, a secretory protein of *H. pylori*, is initially composed in a precursor form with a molecular weight of 140-kDa in the bacteria. The VacA precursor first passes through the bacterial inner membrane with the help of Sec structures and then goes through the β-barrel-type pore of the outer membrane created by the autotransporter of the 40-kDa C-terminal structure protein and is finally secreted as an 88-kDa monomer. In a laboratory condition, VacA, after being purified from the *H. pylori* culture medium, is a water-soluble oligomeric complex or more specifically takes the form of either a hexameric ring or heptameric ring [30]. Therefore, in order to convert the purified VacA proteins to demonstrate virulence when binding to a host cell, either an acidic or alkalic condition should be required to decompose the VacA oligomeric complex into monomers.

5.3.2 *vacA* Gene Diversity

Although almost every *H. pylori* strain possesses the *vacA* gene, the degree of forming capacity of vacuoles in the bacterial culture medium is different for each strain [7, 31]. Various factors have been suggested to address this difference; it may result from the level of *vacA* transcription or the effectiveness of secretion of VacA proteins [32], but the fundamental difference should be attributed to genetic mutation such as nonsense mutation, internal duplication, deletion, or insertion of the *vacA* gene [33]. Consequently, the *vacA* genotype may reflect the difference in the amino acid sequence of VacA proteins, which, in turn, determines the degree of cell activity in response to the different VacA toxins.

VacA-coding genes are known for their characteristics of high mutability. Thus, the *vacA* gene is shown to have several allelic variants with a different base sequence. Specifically, most diversity in the *vacA* gene is concentrated in the signal sequence and mid-region. For the signal sequence, “s1” and “s2” alleles are in the decoding area of the “s-region,” where s1 is further divided into its three subtypes of “s1a,” “s1b,” and “s1c.” The “m-region,“corresponding to the mid-region, has the two alleles of “m1” and “m2.” According to laboratory experiments, the s1-/m1-type strains show high toxicity, whereas the s1-/m2-type strains correspond to low toxicity. In addition, the s2-/m2-type strain does not have toxicity, and the s2-/m1-type strain is highly rare in its presence [6, 34, 35]. In contrast to East Asia, in Latin America, the Middle East, Europe, and Africa, persons infected with *H. pylori* strains with s1 or m1 type are shown to have a higher incidence rate for gastritis and GC compared to *H. pylori* strains with s2 or m2 type [6].

The “intermediate (i)-region” in the *vacA* gene is located between the “s-region” and the “m-region” [36]. Recently, three primary i-region types (i1, i2, and i3) were identified [37]. Although the role of “i-region” regarding the activity of VacA in host cells has not been elucidated much, the i1 type, among the three, is reported to generate the most powerful vacuole

[38]. In addition, the "i-region" has been suggested to be a more promising prognostic factor for GI diseases than the "s-region" or the "m-region" of *vacA* genes, and the i1 type shows a high correlation for gastric adenocarcinoma and gastritis [36, 39]. However, it is acknowledged that there are other research studies with opposing points of view [6]. Currently, the majority of the *vacA* genes isolated in Korea take s1/i1/m1 types [40].

Recently, the "deletion (d)-region" has been found somewhere between the "i-region" and the "m-region" [41]. The "d-region" contains alleles of d1 (no deletion) and d2 (69–81 bp deletion). For *H. pylori* strains isolated in Western countries, d1 is proposed to be a risk factor for gastric mucosal atrophy. Referentially, for the strains isolated in East Asia, s1/i1/d1 types are the most frequently isolated [6, 41].

5.3.3 *vacA* Genotype in Relation to Gastroduodenal Diseases

Similar to the *cagA* genotype, researchers have investigated the relevance between particular *vacA* alleles (i-region, m-region, and s-region) and gastroduodenal diseases. For example, individuals infected with *H. pylori* with the *vacA* s1 allele compared to those infected with the *vacA* s2 allele have been reported to be highly correlated with gastroduodenal diseases [34, 42–44]. In addition, *H. pylori* strains with the *vacA* s1 allele compared to those with the *vacA* s2 allele are known to correspond with a greater risk for gastric adenocarcinoma [45–47]. Similarly, the *vacA* m1 allele, compared to the *vacA* m2 allele, reveals a greater correlation with gastric adenocarcinoma [45–47], gastric epithelial damage as a pre-stage before GC, atrophic gastritis, and intestinal metaplasia [34, 48]. Meanwhile, *H. pylori* strains with both *vacA* s1 and *vacA* m1 alleles are not only attributed to greater numbers of bacteria colonized in gastric mucosa and a greater degree of neutrophil invasion into the mucosa [49] but also lead to a greater correlation with gastric and duodenal ulcers [34, 43] and GCs [42, 47, 50]. However, care should be taken with the aforementioned findings due to controversies associated with the possible confounding factors such as the region and age of the people from which those pathogens have been isolated.

5.3.4 Biological Functions of VacA

VacA can form an ion-conducting channel in the human cell membrane, in which the channel is selectively permeable to negatively charged ions. Thus, VacA monomers bind to the lipid membrane of the cell surface membrane to create a channel. Although the biological effects of VacA seem to depend on the location of the channel being formed, ions and small organic molecules from host cells may be leaked through the channel accordingly. For example, VacA on the cell membrane becomes incorporated into the endosome by endocytosis, in which various ion channels operate to induce water influx in order to form massive vacuoles. In addition, VacA-induced channel formation in the inner membrane of the mitochondria can result in inner membrane depolarization and eventually apoptosis. This kind of apoptosis is observed not only in gastric epithelial cells but also in eosinophil and dendritic cells [51, 52].

VacA proteins also modify the cytoskeleton organization of gastric epithelial cells [53, 54] and subsequently induce autophagy [55, 56]. This autophagy is presumed to take the role of restricting cellular toxicity. In addition, VacA proteins can inhibit T cell activation and proliferation [57] and prevent antigen presentation by B cells [58, 59]. VacA can increase the production of proinflammatory cytokine in mast cells and the infiltration of mast cells [60]. Moreover, VacA can upregulate the calcium influx, the production of reactive oxygen intermediate, and the chemokine expression via NF-κB activation [61]. Furthermore, VacA is capable of affecting neutrophil functions [41, 62]. Based on these reports, immune cells exposed to VacA may result in decreasing antigen recognition and increasing the cellular toxicity that gives rise to GI diseases.

Nevertheless, the underlying mechanisms of VacA and CagA in gastric epithelial cells are different. Since CagA proteins require T4SS, which is to be directly injected into the cytoplasm of gastric epithelial cells, it can only operate on cells colonized with *H. pylori*. On the contrary, VacA, a secretory protein, can function not only on gastric epithelial cells with attached pathogens but also extensively spread to the cells that have not been infected yet. The interaction of VacA proteins and CagA proteins may also exert antagonism to each other. For example, CagA can stimulate the expression of the apoptotic suppressor, Mc11, which inhibits the apoptosis induced by VacA [63–65].

5.4 Outer Membrane Inflammatory Protein (OipA)

The outer membrane proteins refer to proteins that present on the outer membrane of a cell, external to the cytoplasm of *H. pylori*. The space between the inner membrane (cytoplasmic membrane) and outer membrane is called the periplasmic space. This space is to store a variety of materials to be secreted to the outside as well as those from the outside. Recently, more than 32 outer membrane proteins in *H. pylori* have been identified. Most of them are involved in bacterial adhesion [58]. The representative examples include OipA, blood group antigen-binding adhesin (BabA), sialic acid-binding adhesin (SabA), and adherence-associated lipoprotein, of which OipA will be further described.

Among the various OipA functions, the most well-known is the function to induce the expression of interleukin (IL)-8, which stimulates the infiltration of neutrophils. Specifically, OipA and *cag* PAI regulate the activation of the transcription factors such as NF-κB, activator protein-1 (AP-1), and interferon-stimulated responsive element (ISRE)-like element within the IL-8 promoter region in gastric epithelial cells infected with *H. pylori*. Consequently, the activation of these transcription factors results in IL-8 production, leading to the infiltration of neutrophils into gastric mucosa.

In addition to CagA, OipA is involved in β-catenin signal transduction. Thus, the inactivation of the *oipA* gene is reported to decrease the transfer of β-catenin into the nucleus, lowering tumor incidences [3]. Therefore, it is presumed that there may be some type of interaction between OipA and *cag* PAI or between OipA and CagA, which is still to be elucidated. Referentially, the *oipA* gene and *cagA* gene are shown to be highly correlated (correlation coefficient = 0.82) [66]. In addition, *cagA* genes are related to the s-region type of the *vacA* gene and also closely to the *babA* gene [6]. These relationships among virulence factors may raise the possibility of their conveying biological implications and some level of interaction among them. From this viewpoint, rather than assessing the virulence factors individually, it is more appropriate to consider pathogenesis in association with the interaction of these virulence factors integratedly. For the record, the correlation between *cagA*(+), *vacA* s1/i1/m1, *oipA*(+), and *iceA1*(+) strains and the incidences of gastric ulcers are illustrated in research on the strains isolated from South Korea [40].

5.5 Induced by Contact with Epithelium (IceA)

IceA was named to reflect the fact that it is expressed only in the case of *H. pylori* attached to gastric epithelial cells. The *iceA* genes consist of two alleles, *iceA1* and *iceA2* [5]. The sequence of the *iceA1* allele is similar to that of the *nla*IIIR of *Neisseria lactamica*, another pathogen [67], whereas any homology with the *iceA2* allele has not been found with other pathogens. In addition, a correlation of the *iceA* allele with either the *cagA* or *vacA* genes has not been identified.

The *iceA1* allele is highly correlated with peptic ulcers [5]. The expression of *iceA1* genes requires its direct contact with mucosal epithelial cells, after which activation of the genes results in an increase of IL-8 production, leading to acute inflammatory response [67, 68]. Recently, research on strains isolated from South Korea has elucidated the high correlation between the presence of the *iceA1* allele and peptic ulcers [40]. The *iceA2* allele is known to be more prevalent in strains isolated from Western countries than in Japan, which suggests a regional difference [69]. Despite these findings, a correlation between the *iceA2* allele and diseases has not been established.

5.6 Duodenal Ulcer Promoting Gene (*dupA*)

Recently, duodenal ulcer promoting (*dupA*) gene is reported to be associated with *H. pylori*-induced diseases such as duodenal ulcer [69]. The length of the *dupA* open reading frame (ORF) depends on the isolated *H. pylori* strains [70, 71]. Based on the sequence of the putative 5′ regions, it has been proposed that *dupA* has two genotypes such as "long type" and "short type" [69]. However, Hussein et al. classified a *dupA* allele with 1884 bp (short-type) as *dupA1* and a truncated version by the mutations as *dupA2* [72]. Some strains have a presence of mutations in *dupA*, which created a premature stop codon [72–74]. Based on these results, it is possible that *H. pylori* strains with these mutated sequences may not produce intact DupA protein.

The *dupA* gene encodes homologs of VirB4 ATPase, which is thought to be involved in DNA uptake/DNA transfer and protein transfer. The absence of the *dupA* gene was associated with increased susceptibility to low pH [75]. The presence of the *dupA* gene was associated with increased IL-8 production from antral gastric mucosa as well as from gastric epithelial cells. In addition, the *dupA* gene is involved in the activation of transcription factors that bind to the IL-8 promoter, such as NF-κB and AP-1 [75]. These results suggest that the *dupA* gene can contribute to the inflammatory responses to *H. pylori* infection.

Many *vir* genes exist before and after the region of the *dupA* locus [76]. Thus, the genes such as *virB2*, *virB3*, *virB4* (*dupA*), *virB8*, *virB9*, *virB10*, *virB11*, *virD4*, and *virD2* are found in a strain Shi470 [71]. Since these are structurally similar to the T4SS called *cag* PAI and ComB, it is proposed that their gene cluster may be the "third T4SS" [69]. Considering that the complete *dupA* cluster (possessing *dupA* and all adjacent *vir* genes) was significantly associated with duodenal ulcer in the United States [77], a complete *dupA* cluster with intact long-type *dupA* might be a true virulence factor. Therefore, only strains that are intact *dupA* positive and form a novel T4SS might be involved in gastroduodenal diseases [69]. However, the actual function of *dupA* is still not fully understood, and further studies will help to elucidate the clinical importance of *dupA*.

5.7 Other Virulence Factors

In addition to those mentioned above, a variety of *H. pylori* virulence factors have been reported. Although many reports have shown that other virulence factors may play various pathogenic roles in humans, their specific roles of *H. pylori* have not yet been clarified. Therefore, more research should be needed on the functions listed below in the future.

5.7.1 Shape Switch

The transformation of *H. pylori* from the spiral form to the coccoid form occurs under hostile environmental conditions. For example, in environmental conditions such as an insufficient supply of nutrients, desiccation, lack of protection against oxygen, and exposure to antimicrobial agents, *H. pylori* can survive by switching its shape from spiral to coccoid form [78]. They are often referred to as the spiral viable culturable form (SVCF), whereas the coccoid form is viable but non-culturable (CVNCF or VBNC). CVNCF cannot be detected by a simple culture method, but electron microscopy and molecular techniques are usually used to detect it [79]. Morphological transformation into coccoid form by *H. pylori* is accompanied by a decrease in cell size and a drastic decrease in metabolic activity, leading to survival under harsh environments. CVNCF can persist for a prolonged time and cause severe damage to the gastric mucosa [80]. In addition, the coccoid form can revert to the spiral morphology [81].

5.7.2 High-Temperature Requirement A (HtrA) and Heat-Shock Proteins (Hsps)

High-temperature requirement A (HtrA) is heat shock-induced serine protease and a chaperone protecting protein expressed in human and prokaryotic cells. This protein is resistant to heat and pH [78]. All *H. pylori* strains have htrA genes [82, 83] and secrete HtrA proteins in the extracellular space, where the proteins act as a specific E-cadherin protease that effectively destroys adherence junctions in gastric epithelial cells, allowing the translocation of effector protein [78]. As the virulence factor, HtrA proteins destroy epithelial barrier function, allowing persistent *H. pylori* colonization and pathogenesis. One study showed that the presence of htrA-171 polymorphism resulted in a higher rate of GC [84]. However, more studies are still needed to elucidate whether htrA genetic polymorphism has a significant association with the development of GC.

Heat shock proteins (Hsps) constitute a family of proteins that act as molecular chaperones, being responsible for the maintenance of proper structural and functional properties of the cellular proteins. *H. pylori* has a variety of Hsps such as HspB (Hsp60), HspA (Hsp10), and Hsp70. These Hsps may be involved in (1) enhancing adherence to epithelial surfaces, (2) urease activation, (3) controlling apoptosis and autophagy, (4) maintaining the structure and properties of the effector proteins, (5) protecting the cell from reactive oxygen species (ROS), and (6) inducing the production and release of IL-8, TNF-α, and COX-2 [78, 85].

5.7.3 Arginase

Arginases are Mn2+-metalloenzymes belonging to the ureohydrolase family that catalyzes the hydrolysis of L-arginine into L-ornithine and urea during the urea cycle [86]. *H. pylori* arginase is encoded by the RocF gene. The bacterial arginase proteins are reported in (1) preventing bacterial killing and T-cell proliferation, (2) impairing immune responses, (3) stimulating host cell apoptosis, (4) and bacterial colonization because it can provide acid resistance in the gastric microenvironment [78, 85].

5.7.4 Catalase and Superoxidase Dismutase (SOD)

Catalase is an enzyme that converts hydrogen peroxide (H_2O_2) into water (H_2O) and molecular oxygen (O_2) and is one of the most abundant proteins. Catalase levels are estimated at 4–5% of *H. pylori* total protein content, and it is one of the most highly expressed proteins in *H. pylori* strains obtained from the gastric mucosa [87–89]. *H. pylori* catalase may be involved in (1) induction of mutagenesis, (2) inflammation, (3) protection of *H. pylori* from complement-mediated killing, and (4) maintenance of bacterial survival at the cell surface of the phagocytes and in the macrophage phagosomes, leading to protection *H. pylori* from phagocytosis [78, 85].

Superoxide dismutase (SOD) is one of the *H. pylori* enzymes and facilitates the dismutation of superoxide to oxygen, preventing excessive amounts of toxic superoxide free radicals. *H. pylori* SOD may (1) facilitate bacterial colonization, (2) protect from reactive oxygen species (ROS), and (3) inhibit the production of pro-inflammatory cytokines [90–93].

5.7.5 Cholesteryl α-Glucosyltransferase

Cholesteryl α-glucosyltransferase is a quite poorly characterized enzyme of *H. pylori* that is involved in the formation of cholesteryl α-glucoside (αCGL) by adding α-glucosyl to the cholesterol of the host cellular membranes. *H. pylori* does not synthesize cholesterol on its own but extracts it from the plasma membranes of the host cells; αCgT is involved in the subsequent glycosylation of the cholesterol. αCgT is produced by *H. pylori* in an inactive form, which becomes activated after binding to the host cell membrane [94]. These proteins may be involved in (1) prevention of *H. pylori* from the phagocytosis and immune responses, (2) control of the responses from the $CD4^+$ T-cells, IL-4, and IFN-γ pathways, (3) stimulation of IL-8 secretion, (4) bacterial growth, survival, and antibiotic resistance, and (5) inhibition of the autophagosome-lysosome fusion [85, 95].

5.8 Conclusion

Many studies have been conducted to elucidate pathological mechanisms regarding the components originating from and materials secreted from *H. pylori* associated with human GI diseases. So far, these researches suggest that the virulence factors, including CagA, VacA, OipA, IceA, and *dupA*, play a key role. In addition, various types of virulence factors such as shape switch A, HtrA, Hsps, arginase, catalase, SOD, and cholesteryl α-glucosyltransferase have been reported. Nevertheless, it should be acknowledged that the aforementioned pathological mechanisms of these virulence factors in *H. pylori* infection still do not seem to fit all the pieces together. Above all, the fact that *H. pylori* strains contain more than 1600 genes opens the possibility of finding additional new pathogenic genes in the future. Moreover, host factors (e.g., IL-1 genetic polymorphism) are expected to play a substantial role, more of which will be discussed in the next chapter. Whole genome analyses may be useful for the investigation of genetic factors that are related to differences in virulence among strains. Therefore, larger amounts of data will become available in the near future, and other important novel virulence factors can be discovered.

References

1. Kim JM. Current researches on the virulence factors of *Helicobacter pylori*. Korean J Gastroenterol. 2003;41:77–86.
2. Bridge DR, Merrell DS. Polymorphism in the *Helicobacter pylori* CagA and VacA toxins and disease. Gut Microbes. 2013;4:101–17.
3. Franco AT, Johnston E, Krishna U, Yamaoka Y, Israel DA, Nagy TA, et al. Regulation of gastric carcinogenesis by *Helicobacter pylori* virulence factors. Cancer Res. 2008;68:379–87.
4. Ohnishi N, Yuasa H, Tanaka S, Sawa H, Miura M, Matsui A, et al. Transgenic expression of *Helicobacter pylori* CagA induces gastrointestinal and hematopoietic neoplasms in mouse. Proc Natl Acad Sci U S A. 2008;105:1003–8.
5. van Doorn LJ, Figueiredo C, Sanna R, Plaisier A, Schneebergeret P, de Boer W, et al. Clinical relevance of the cagA, vacA, and iceA status of *Helicobacter pylori*. Gastroenterology. 1998;115:58–66.
6. Yamaoka Y. Mechanisms of disease: *Helicobacter pylori* virulence factors. Nat Rev Gastroenterol Hepatol. 2010;7:629–41.
7. Kim JM, Kim JS, Jung HC, Song IS, Kim CY. Virulence factors of *Helicobacter pylori* in Korean isolates do not influence proinflammatory cytokine gene expression and apoptosis in human gastric epithelial cells, nor do these factors influence the clinical outcome. J Gastroenterol. 2000;35:898–906.
8. Israel DA, Salama N, Krishna U, Rieger UM, Atherton JC, Falkow S, et al. *Helicobacter pylori* genetic diversity within the gastric niche of a single human host. Proc Natl Acad Sci U S A. 2001;98:14625–30.
9. Noto JM, Peek RM Jr. The *Helicobacter pylori* cag pathogenicity island. Methods Mol Biol. 2012;921:41–50.
10. Camorlinga-Ponce M, Romo C, Gonzalez-Valencia G, MunoZ O, Torres J. Topographical localisation of cagA positive and cagA negative *Helicobacter pylori* strains in the gastric mucosa; an in situ hybridisation study. J Clin Pathol. 2004;57:822–8.
11. Tegtmeyer N, Wessler S, Backert S. Role of the cag-pathogenicity island encoded type IV secretion system in *Helicobacter pylori* pathogenesis. FEBS J. 2011;278:1190–202.
12. Kwok T, Zabler D, Urman S, Rohde M, Hartig R, Wessler S, et al. Helicobacter exploits integrin for type IV secretion and kinase activation. Nature. 2007;449:862–6.
13. Jimenez-Soto LF, Kutter S, Sewald X, Ertl C, Weiss E, Kapp U, et al. *Helicobacter pylori* type IV secretion apparatus exploits beta1 integrin in a novel RGD-independent manner. PLoS Pathog. 2009;5:e1000684.
14. Hatakeyama M. Oncogenic mechanisms of the *Helicobacter pylori* CagA protein. Nat Rev Cancer. 2004;4:688–94.
15. Argent RH, Kidd M, Owen RJ, Thomas RJ, Limb MC, Atherton JC. Determinants and consequences of different levels of CagA phosphorylation for clinical isolates of *Helicobacter pylori*. Gastroenterology. 2004;127:514–23.
16. Azuma T, Yamakawa A, Yamazaki S, Fukuta K, Ohtani M, Ito Y, et al. Correlation between variation of the 3′ region of the cagA gene in *Helicobacter pylori* and disease outcome in Japan. J Infect Dis. 2002;186:1621–30.
17. Xia Y, Yamaoka Y, Zhu Q, Matha I, Gao X. A comprehensive sequence and disease correlation analyses for

the C-terminal region of CagA protein of *Helicobacter pylori*. PLoS One. 2009;4:e7736.

18. Yamaoka Y, Kodama T, Kashima K, Graham DY, Sepulveda AR. Variants of the 3′ region of the cagA gene in *Helicobacter pylori* isolates from patients with different *H. pylori*-associated diseases. J Clin Microbiol. 1998;36:2258–63.
19. Poppe M, Feller SM, Romer G, Wessler S. Phosphorylation of *Helicobacter pylori* CagA by c-Abl leads to cell motility. Oncogene. 2007;26:3462–72.
20. Selbach M, Moese S, Hauck CR, Meyer TF, Backert S. Src is the kinase of the *Helicobacter pylori* CagA protein in vitro and in vivo. J Biol Chem. 2002;277:6775–8.
21. Tammer I, Brandt S, Hartig R, Konig W, Backert S. Activation of Abl by *Helicobacter pylori*: a novel kinase for CagA and crucial mediator of host cell scattering. Gastroenterology. 2007;132:1309–19.
22. Backert S, Tegtmeyer N, Selbach M. The versatility of *Helicobacter pylori* CagA effector protein functions: the master key hypothesis. Helicobacter. 2010;15:163–76.
23. Selbach M, Paul FE, Brandt S, Guye P, Daumke O, Backert S, et al. Host cell interactome of tyrosine-phosphorylated bacterial proteins. Cell Host Microbe. 2009;5:397–403.
24. Ren S, Higashi H, Lu H, Azuma T, Hatakeyama M. Structural basis and functional consequence of *Helicobacter pylori* CagA multimerization in cells. J Biol Chem. 2006;281:32344–52.
25. Saadat I, Higashi H, Obuse C, Umeda M, Murata-Kamiya N, Saito Y, et al. *Helicobacter pylori* CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. Nature. 2007;447:330–3.
26. Umeda M, Murata-Kamiya N, Saito Y, Ohba Y, Takahashi M, Hatakeyama M. *Helicobacter pylori* CagA causes mitotic impairment and induces chromosomal instability. J Biol Chem. 2009;284:22166–72.
27. Nesic D, Miller MC, Quinkert ZT, Stein M, Chait BT, Stebbins CE. *Helicobacter pylori* CagA inhibits PAR1-MARK family kinases by mimicking host substrates. Nat Struct Mol Biol. 2010;17:130–2.
28. Suzuki M, Mimuro H, Kiga K, Fukumatsu M, Ishijima N, Nagai S, et al. *Helicobacter pylori* CagA phosphorylation-independent function in epithelial proliferation and inflammation. Cell Host Microbe. 2009;5:23–34.
29. Tsang YH, Lamb A, Romero-Gallo J, Huang B, Ito K, Peek RM Jr, et al. *Helicobacter pylori* CagA targets gastric tumor suppressor RUNX3 for proteasome-mediated degradation. Oncogene. 2010;29:5643–50.
30. Gonzalez-Rivera C, Gangwer KA, McClain MS, Eli IM, Chambers MG, Ohi MD, et al. Reconstitution of *Helicobacter pylori* VacA toxin from purified components. Biochemistry. 2010;49:5743–52.
31. Cover TL, Blaser MJ. *Helicobacter pylori* in health and disease. Gastroenterology. 2009;136:1863–73.
32. Forsyth MH, Atherton JC, Blaser MJ, Cover TL. Heterogeneity in levels of vacuolating cytotoxin gene (vacA) transcription among *Helicobacter pylori* strains. Infect Immun. 1998;66:3088–94.
33. Ito Y, Azuma T, Ito S, Suto H, Miyaji H, Yamazaki Y, et al. Full-length sequence analysis of the vacA gene from cytotoxic and noncytotoxic *Helicobacter pylori*. J Infect Dis. 1998;178:1391–8.
34. Atherton JC, Cao P, Peek RM Jr, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem. 1995;270:17771–7.
35. Letley DP, Lastovica A, Louw JA, Hawkey CJ, Atherton JC. Allelic diversity of the *Helicobacter pylori* vacuolating cytotoxin gene in South Africa: rarity of the vacA s1a genotype and natural occurrence of an s2/m1 allele. J Clin Microbiol. 1999;37:1203–5.
36. Rhead JL, Letley DP, Mohammad M, Hussein N, Mohagheghi MA, Eshagh Hosseini M, et al. A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. Gastroenterology. 2007;133:926–36.
37. Chung C, Olivares A, Torres E, Yilmaz O, Cohen H, Perez-Perez G. Diversity of VacA intermediate region among *Helicobacter pylori* strains from several regions of the world. J Clin Microbiol. 2010;48:690–6.
38. Kim IJ, Blanke SR. Remodeling the host environment: modulation of the gastric epithelium by the *Helicobacter pylori* vacuolating toxin (VacA). Front Cell Infect Microbiol. 2012;2:37.
39. Basso D, Zambon CF, Letley DP, Stranges A, Marchet A, Rhead JL, et al. Clinical relevance of *Helicobacter pylori* cagA and vacA gene polymorphisms. Gastroenterology. 2008;135:91–9.
40. Kim JY, Kim N, Nam RH, Suh JH, Chang H, Lee JW, et al. Association of polymorphisms in virulence factor of *Helicobacter pylori* and gastroduodenal diseases in South Korea. J Gastroenterol Hepatol. 2014;29:984–91.
41. Ogiwara H, Sugimoto M, Ohno T, Vilaichone RK, Mahachai V, Graham DY, et al. Role of deletion located between the intermediate and middle regions of the *Helicobacter pylori* vacA gene in cases of gastroduodenal diseases. J Clin Microbiol. 2009;47:3493–500.
42. Kidd M, Lastovica AJ, Atherton JC, Louw JA. Heterogeneity in the *Helicobacter pylori* vacA and cagA genes: association with gastroduodenal disease in South Africa? Gut. 1999;45:499–502.
43. Rudi J, Kolb C, Maiwald M, Kuck D, Sieg A, Galle PR, et al. Diversity of *Helicobacter pylori* vacA and cagA genes and relationship to VacA and CagA protein expression, cytotoxin production, and associated diseases. J Clin Microbiol. 1998;36:944–8.
44. van Doorn LJ, Figueiredo C, Megraud F, Pena S, Midolo P, Queiroz DM, et al. Geographic distribution of vacA allelic types of *Helicobacter pylori*. Gastroenterology. 1999;116:823–30.
45. Ashour AA, Magalhaes PP, Mendes EN, Collares GB, de Gusmao VR, Queiroz DM, et al. Distribution of vacA genotypes in *Helicobacter pylori* strains

isolated from Brazilian adult patients with gastritis, duodenal ulcer or gastric carcinoma. FEMS Immunol Med Microbiol. 2002;33:173–8.

46. Figueiredo C, Machado JC, Pharoah P, Seruca R, Sousa S, Carvalho R, et al. *Helicobacter pylori* and interleukin 1 genotyping: an opportunity to identify high-risk individuals for gastric carcinoma. J Natl Cancer Inst. 2002;94:1680–7.
47. Miehlke S, Kirsch C, Agha-Amiri K, Gunther T, Lehn N, Malfertheiner P, et al. The *Helicobacter pylori* vacA s1, m1 genotype and cagA is associated with gastric carcinoma in Germany. Int J Cancer. 2000;87:322–7.
48. Nogueira C, Figueiredo C, Carneiro F, Gomes AT, Barreira R, Figueira P, et al. *Helicobacter pylori* genotypes may determine gastric histopathology. Am J Pathol. 2001;158:647–54.
49. Hofman VJ, Moreilhon C, Brest PD, Lassalle S, Le Brigand K, Sicard D, et al. Gene expression profiling in human gastric mucosa infected with *Helicobacter pylori*. Mod Pathol. 2007;20:974–89.
50. Basso D, Navaglia F, Brigato L, Piva MG, Toma A, Greco E, et al. Analysis of *Helicobacter pylori* vacA and cagA genotypes and serum antibody profile in benign and malignant gastroduodenal diseases. Gut. 1998;43:182–6.
51. Kim JM, Kim JS, Lee JY, Sim YS, Oh YK, Yoon HJ, et al. Dual effects of *Helicobacter pylori* vacuolating cytotoxin on human eosinophil apoptosis in early and late periods of stimulation. Eur J Immunol. 2010;40:1651–62.
52. Kim JM, Kim JS, Kim N, Ko SH, Jeon JI, Kim YJ. *Helicobacter pylori* vacuolating cytotoxin induces apoptosis via activation of endoplasmic reticulum stress in dendritic cells. J Gastroenterol Hepatol. 2015;30:99–108.
53. Gupta VR, Wilson BA, Blanke SR. Sphingomyelin is important for the cellular entry and intracellular localization of *Helicobacter pylori* VacA. Cell Microbiol. 2010;12:1517–33.
54. Papini E, Zoratti M, Cover TL. In search of the *Helicobacter pylori* VacA mechanism of action. Toxicon. 2001;39:1757–67.
55. Greenfield LK, Jones NL. Modulation of autophagy by *Helicobacter pylori* and its role in gastric carcinogenesis. Trends Microbiol. 2013;21:602–12.
56. Terebiznik MR, Raju D, Vazquez CL, Torbricki K, Kulkami R, Blanke SR, et al. Effect of *Helicobacter pylori*'s vacuolating cytotoxin on the autophagy pathway in gastric epithelial cells. Autophagy. 2009;5:370–9.
57. Schmees C, Gerhard M, Treptau T, Voland P, Schwendy S, Rad R, et al. VacA-associated inhibition of T-cell function: reviewed and reconsidered. Helicobacter. 2006;11:144–6.
58. Lu H, Yamaoka Y, Graham DY. *Helicobacter pylori* virulence factors: facts and fantasies. Curr Opin Gastroenterol. 2005;21:653–9.
59. Molinari M, Salio M, Galli C, Norais N, Rappuoli R, LanZavecchia A, et al. Selective inhibition of Ii-dependent antigen presentation by *Helicobacter pylori* toxin VacA. J Exp Med. 1998;187:135–40.
60. Supajatura V, Ushio H, Wada A, Yahiro K, Okumura K, Ogawa H, et al. Cutting edge: VacA, a vacuolating cytotoxin of *Helicobacter pylori*, directly activates mast cells for migration and production of proinflammatory cytokines. J Immunol. 2002;168:2603–7.
61. Kim JM, Kim JS, Lee JY, Kim YJ, Youn HJ, Kim IY, et al. Vacuolating cytotoxin in *Helicobacter pylori* water-soluble proteins upregulates chemokine expression in human eosinophils via Ca2+ influx, mitochondrial reactive oxygen intermediates, and NF-kappaB activation. Infect Immun. 2007;75:3373–81.
62. Brest P, Hofman V, Lassalle S, Cesaro A, Ricci V, Selva E, et al. Human polymorphonuclear leukocytes are sensitive in vitro to *Helicobacter pylori* VacA toxin. Helicobacter. 2006;11:544–55.
63. Akada JK, Aoki H, Torigoe Y, Kitagawa T, Kurazono H, Hoshida H, et al. *Helicobacter pylori* CagA inhibits endocytosis of cytotoxin VacA in host cells. Dis Model Mech. 2010;3:605–17.
64. Mimuro H, Suzuki T, Nagai S, Rieder G, Suzuki M, Nagai T, et al. *Helicobacter pylori* dampens gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach. Cell Host Microbe. 2007;2:250–63.
65. Oldani A, Cormont M, Hofman V, Chiozzi V, Oregioni O, Canonici A, et al. *Helicobacter pylori* counteracts the apoptotic action of its VacA toxin by injecting the CagA protein into gastric epithelial cells. PLoS Pathog. 2009;5:e1000603.
66. Yamaoka Y, Kikuchi S, el-Zimaity HM, Gutierrez O, Osato MS, Graham DY. Importance of *Helicobacter pylori* oipA in clinical presentation, gastric inflammation, and mucosal interleukin 8 production. Gastroenterology. 2002;123:414–24.
67. Peek RM Jr, Thompson SA, Donahue JP, Tham KT, Atherton JC, Blaser MJ, et al. Adherence to gastric epithelial cells induces expression of a *Helicobacter pylori* gene, *iceA*, that is associated with clinical outcome. Proc Assoc Am Physicians. 1998;110:531–44.
68. Blaser MJ. Heterogeneity of *Helicobacter pylori*. Eur J Gastroenterol Hepatol. 2012;9 (Suppl 1):S3–6.
69. Shiota S, Suzuki R, Yamaoka Y. The significance of virulence factors in *Helicobacter pylori*. J Dig Dis. 2013;14:341–9.
70. Kersulyte D, Kalia A, Gilman RH, Mendez M, Herrera P, Cabrera L, et al. *Helicobacter pylori* from Peruvian Amerindians: traces of human migrations in strains from remote Amazon, and genome sequence of an Amerind strain. PLoS One. 2010;5:e15076.
71. Kersulyte D, Lee W, Subramaniam D, Anant S, Herrera P, Cabrera L, et al. Helicobacter pylori's plasticity zones are novel transposable elements. PLoS One. 2009;4:e6859.
72. Hussein NR, Argent RH, Marx CK, Patel SR, Robinson K, Atherton JC. *Helicobacter pylori* dupA is polymorphic, and its active form induces proinflammatory cytokine secretion by mononuclear cells. J Infect Dis. 2010;202:261–9.

73. Gomes LI, Rocha GA, Rocha AM, Soares TF, Oliveira CA, Bittencourt PF, et al. Lack of association between *Helicobacter pylori* infection with dupA-positive strains and gastroduodenal diseases in Brazilian patients. Int J Med Microbiol. 2008;298:223–30.
74. Queiroz DM, Rocha GA, Rocha AM, Moura SB, Saraiva IE, Gomes LI, et al. *dupA* polymorphisms and risk of *Helicobacter pylori*-associated diseases. Int J Med Microbiol. 2011;301:225–8.
75. Lu H, Hsu PI, Graham DY, Yamaoka Y. Duodenal ulcer promoting gene of *Helicobacter pylori*. Gastroenterology. 2005;128:833–48.
76. Yamaoka Y. Roles of the plasticity regions of *Helicobacter pylori* in gastroduodenal pathogenesis. J Med Microbiol. 2008;57:545–53.
77. Jung SW, Sugimoto M, Shiota S, Graham DY, Yamaoka Y. The intact dupA cluster is a more reliable *Helicobacter pylori* virulence marker than dupA alone. Infect Immun. 2012;80:381–7.
78. Sharndama HC, Mba IE. *Helicobacter pylori*: an up-to-date overview on the virulence and pathogenesis mechanisms. Braz J Microbiol. 2022;53:33–50.
79. Elhahriri M, Hanza D, Elhew R, Hamza E. Occurrence of cagA+ vacA s1a m1 i1 *Helicobacter pylori* in farm animals in Egypt and ability to survive in experimentally contaminated UHT milk. Sci Rep. 2018;8:1–3.
80. Krzyżek P, Grande R. Transformation of *Helicobacter pylori* into Coccoid forms as a challenge for research determining activity of antimicrobial substances. Pathogens. 2020;9:184.
81. Andersen AP, Elliott DA, Lawson M, Barland P, Hatcher VB, Puszkin EG. Growth and morphological transformations of *Helicobacter pylori* in broth media. J Clin Microbiol. 1997;35:2918.
82. Chang WL, Yeh YC, Sheu BS. The impacts of *H. pylori* virulence factors on the development of gastroduodenal diseases. J Biomed Sci. 2018;25:68.
83. Tegtmeyer N, Moodley Y, Yamaoka Y, Pernitzsch SR, Schmidt V, Traverso FR, et al. Characterisation of worldwide *Helicobacter pylori* strains reveals genetic conservation and essentiality of serine protease HtrA. Mol Microbiol. 2016;99:925–44.
84. Yeh YC, Kuo HY, Chang WL, Yang HB, Lu CC, Cheng HC, et al. *H. pylori* isolates with amino acid sequence polymorphisms as presence of both HtrA-L171 & CagL-Y58/E59 increase the risk of gastric cancer. J Biomed Sci. 2019;26:4.
85. Baj J, Forma A, Sitarz M, Portincasa P, Garruti G, Krasowska D, et al. *Helicobacter pylori* virulence factors-mechanisms of bacterial pathogenicity in the gastric microenvironment. Cells. 2020;10:27.
86. Mendz GL, Hazell SL. The urea cycle of *Helicobacter pylori*. Microbiology. 1996;142:2959–67.
87. Harris AG, Hazell SL. Localisation of *Helicobacter pylori* catalase in both the periplasm and cytoplasm, and its dependence on the twin-arginine target protein, KapA, for activity. FEMS Microbiol Lett. 2003;229:283–9.
88. Mahawar M, Tran V, Sharp JS, Maier RJ. Synergistic roles of *Helicobacter pylori* methionine sulfoxide reductase and GroEL in repairing oxidant-damaged catalase. J Biol Chem. 2011;286:19159–69.
89. Boonjakuakul JK, Syvanen M, Suryaprasad A, Bowlus CL, Solnick JV. Transcription profile of *Helicobacter pylori* in the human stomach reflects its physiology in vivo. J Infect Dis. 2004;190:946–56.
90. Morishita K, Takeuchi H, Morimoto N, Shimamura T, Kadota Y, Tsuda M, et al. Superoxide dismutase activity of *Helicobacter pylori* per se from 158 clinical isolates and the characteristics. Microbiol Immunol. 2012;56:262–72.
91. Švagelj D, Terzić V, Dovhanj J, Švagelj M, Cvrković M, Švagelj I. Superoxide dismutases in chronic gastritis. APMIS. 2016;124:252–6.
92. Negovan A, Iancu M, Tripon F, Crauciuc A, Mocan S, Bănescu C. The CAT-262 C> T, MnSOD Ala16Val, GPX1 Pro-198Leu polymorphisms related to oxidative stress and the presence of gastric lesions. J Gastrointestin Liver Dis. 2018;27:371–8.
93. Stent A, Every AL, Chionh YT, Ng GZ, Sutton P. Superoxide dismutase from *Helicobacter pylori* suppresses the production of pro-inflammatory cytokines during in vivo infection. Helicobacter. 2018;23:e12459.
94. Hoshino H, Tsuchida A, Kametani K, Mori M, Nishizawa T, Suzuki T, et al. Membrane-associated activation of cholesterol α-glucosyltransferase, an enzyme responsible for biosynthesis of cholesteryl-α-D-glucopyranoside in *Helicobacter pylori* critical for its survival. J Histochem Cytochem. 2011;59:98–105.
95. Shibayama K, Wachino JI, Arakawa Y, Saidijam M, Rutherford NG, Henderson PJ. Metabolism of glutamine and glutathione via γ-glutamyltranspeptidase and glutamate transport in *Helicobacter pylori*: possible significance in the pathophysiology of the organism. Mol Microbiol. 2007;64:396–406.

H. pylori Virulence Factors: Genetic Polymorphism and Disease

6

Young Sun Kim

Abstract

Helicobacter pylori (*H. pylori*) virulence factors are important for clarifying the role of *H. pylori* in the regional differences in gastric cancer distribution and the pathogenesis of clinically significant diseases such as gastric cancer or peptic ulcer. The polymorphisms in host genes encoding the immune effector proteins play important roles in the inter-individual variations in clinical outcomes since they directly add to the susceptibility of an individual to *H. pylori* infection and related diseases. Genetic polymorphism of *H. pylori* virulence factors differs by geographic region, in which East-Asian-type *cagA* is known to be more virulent than Western type. As there are more repetitions of the Glu-Pro-Ile-Tyr-Ala (EPIYA)-C segment in *cagA*, *H. pylori* becomes more virulent, which is associated with gastric cancers in the West. Between genotype m1 and m2 of the *vacA* middle region, m1 is more virulent, which is thought to be the cause of the increased prevalence rate of gastric cancers in many East-Asian regions. If all the studies to date are put together, *cagA*, *vacA*, *oipA,* and *babA* are the factors associated with gastric cancer, and *dupA* can be considered to be an important virulent factor for duodenal ulcer, but because different studies have shown different results and, particularly, results were different by geographic region, more research is needed.

Keywords

Helicobacter pylori · Virulence factor · Genetic polymorphism

6.1 Introduction

Helicobacter pylori (*H. pylori*) has a genome of 1600 genes, which are well conserved, but its variants are very diverse and complex in terms of combination and structure of the genes by region depending on the physiological and ecological changes in the strains and hosts, which makes an interesting subject as a model for adaptation and evolution of microorganisms [1, 2].

H. pylori strains have evolved continuously within the human body when mankind left Africa and moved to America and Oceania approximately 58,000 years ago, and it is predicted that the virulence of *H. pylori* has been changed along [1, 3] (Fig. 6.1). For example, a genetic analysis of a strain isolated from Peruvians in South America demonstrated that while *cag* pathogenicity island (PAI) gene of Peruvian natives did not contain cytotoxin-associated gene A (*cagA*) gene, *cagA* gene of Europeans was newly inserted after the arrival of Europeans. In addition, by a

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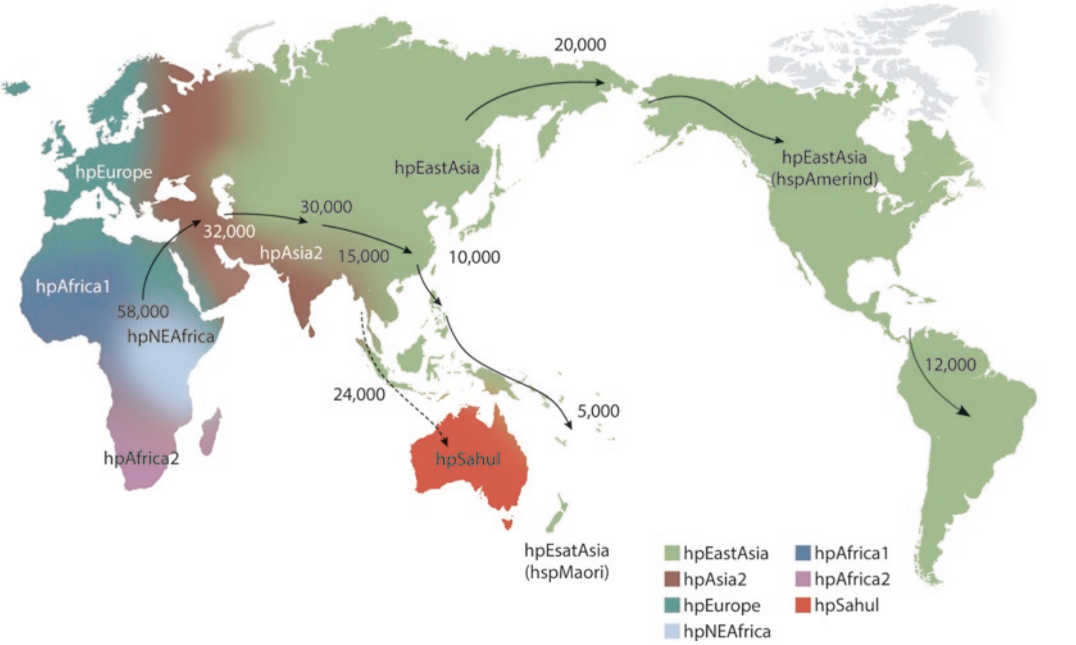

Fig. 6.1 Distribution of *H. pylori* genotypes before Columbus found the New World and human migration to America and Oceania began. There are seven modern *H. pylori* population types: hpEurope, hpEastAsia, hpAfrica1, hpAfrica2, hpAsia2, hpNEAfrica, and hpSahul. hpEurope includes almost all *H. pylori* strains isolated from ethnic Europeans, including people from countries colonized by Europeans. Most *H. pylori* isolates from East Asia belong to hpEastAsia, which includes hspMaori (Polynesians, Melanesians, and native Taiwanese), hspAmerind (American Indians), and hspEAsia (East Asia) subpopulations. hpAsia2 strains are isolated in South, Southeast, and Central Asia; hpAfrica1 in West Africa, South Africa, and African Americans. hpNEAfrica is predominantly made up of isolates from Northeast Africa. hpAfrica2 is very distinct from any other type and has currently only been isolated in South Africa. hpSahul is a novel group specific to *H. pylori* strains isolated from Australian Aborigines and Highlanders of New Guinea. *H. pylori* is predicted to have spread from East Africa over the same time period as anatomically modern humans (~58,000 years ago) and has remained intimately associated with their human hosts ever since. Estimated global patterns of *H. pylori* migration are indicated by *arrows*, and the *numbers* show the estimated time since they migrated (years ago). The *broken arrow* indicates an unconfirmed migration pattern (Adapted from Yamaoka et al. [4])

comparison of *cagA* DNA sequences, it was also confirmed that genes of Indo-Aryans and Neolithic man in the Crescent region coexist in the Indian strain. These genetic diversities of virulence factors including *cagA* and vacuolating cytotoxin (*vacA*) depending on the region suggest a possibility that the pathogenicity of *H. pylori* is an acquired characteristic for survival and adaptation through the process of evolution and transfer [3, 5, 6].

H. pylori live in the human stomach for a long time, but most of those infected do not have symptoms and only some of those infected are taken with diseases, which are not necessarily the same diseases. Thus, the reason why those infected show different results is because bacterial factors, in particular virulence factors, as well as host and environmental factors play an important role. Although genetic studies of virulence factors have been actively conducted based on the already known DNA sequence of *H. pylori*, crucial virulence factor genes responsible for inducing diseases have not been fully established yet, which can be explained by a high degree of variations in the DNA sequences of each *H. pylori* (genetic polymorphism). Such diverse variations are caused by point mutation, substitution, insertion, or deletion, and occasionally several strains of *H. pylori* with different genetic backgrounds are observed in a single

Table 6.1 Association of *Helicobacter pylori* virulence factors with host responses and disease outcome

Virulence factor	Colonization/cell damage	Inflammation/immunity	Disease outcome
cag PAI	Increased bacterial density	Increased production of cytokines and antimicrobials Infiltration of immune cells	Gastritis Peptic ulcer Gastric cancer
CagA	Disruption of epithelial junctions Epithelial motility and scattering	Cytokine production Up-regulation of oncogenic proteins	Atrophic gastritis Gastric cancer
VacA	Colonization Epithelial permeability Epithelial erosion and necrosis Vacuolation Apoptosis	Disruption of antigen processing Inhibition of T-cell proliferation	Ulceration
OipA	Adherence to epithelial cells Colonization	Cytokine production	Dysplasia Gastric cancer Duodenal ulcer
DupA		Cytokine production	Duodenal ulcer Decreased gastric cancer

Modified from Allison and Ferrero [14]
cag PAI cag pathogenicity island, *CagA* cytotoxin-associated gene A, *VacA* vacuolating cytotoxin, *OipA* outer inflammatory protein, *DupA* duodenal ulcer promoting gene

individual. Through these observations, it is thought that not only endogenous mutations but also chromosomal rearrangements or recombination occur between each strain [1]. So far, there have been many studies to examine the relationship between diseases and known virulence factors, such as CagA, VacA, induced by contact with epithelium (IceA), outer inflammatory protein (OipA), duodenal ulcer promoting gene (*dupA*), and blood group A antigen-binding adhesion (BabA) [4, 7–14] (Table 6.1). In this chapter, we will discuss the relationship between the genetic polymorphism of these virulence factors and gastroduodenal diseases with geographic differences.

6.2 Cytotoxin-Associated Gene A (*cagA*)

CagA is the most studied virulence factor of *H. pylori*, which is located at one end of the *cag* PAI. The *cag* PAI encodes a type IV secretion system, responsible for the injection of the CagA protein into the host cells [15–17].

CagA-producing strains are reported to be associated with severe clinical outcomes, especially in Western countries. Approximately, 60–70% of isolated *H. pylori* strains from Western countries are known to be positive for *cagA*, which causes more severe inflammatory reactions with increased interleukin (IL)-8 production. It has been reported that individuals infected with *cagA*-positive strains of *H. pylori* are at a higher risk of peptic ulcer or gastric cancer than those infected with *cagA*-negative strains [7, 18]. The studies conducted in Western countries showed that the prevalence of *cagA* antibodies was significantly higher in peptic ulcer patients or duodenal ulcer patients compared to control, that is, 100% for peptic ulcer disease, 85–100% for duodenal ulcer, and 30–60% for control [8, 9].

A study using Western blotting method for serological detection of antibodies against *cagA* reported that serum antibodies to *cagA* were detected more frequently in gastric carcinoma patients (91%) than control (72%) [19].

A cohort study (mean follow-up period of 11.5 years) for 58 subjects infected with *H. pylori* showed that infection with *cagA*-positive *H. pylori* strains is associated with an increased risk for the eventual development of atrophic gastritis and intestinal metaplasia [20]. However, it is difficult to determine the importance of *cagA* in clinical outcomes in East-Asian countries including Korea because nearly all *H. pylori* strains possess *cagA* [9, 10, 12, 13].

Several studies have attempted to investigate the association between *cagA* status and the development of gastric mucosa-associated lymphoid tissue (MALT) lymphoma, but the results are unclear [21]. A recent systematic review and meta-analysis showed that no relationship between *cagA* status and gastric MALT lymphoma in Western countries. However, interestingly, the study showed an inverse association between *cagA* status and gastric MALT lymphoma risk in the Asian population (OR 0.104; 95% CI, 0.036–0.307). In addition, a meaningful association was observed between *cagA* status and diffuse large B-cell lymphoma (OR 6.43; 95% CI, 2.45–16.84), suggesting that translocated *cagA* antigen into the B cells plays a crucial role in the development of diffuse large B-cell lymphoma [21]. More researches are needed to confirm these results.

6.2.1 *cagA* Type: Western Versus East Asian

There has been an increasing tendency in the last decade to explain the higher incidence of gastric cancer in East Asia using the concept of East-Asian-type *cagA* and Western-type *cagA* [4]. There are different numbers of repeat sequences located in the 3′ region of the *cagA* gene of different *H. pylori* strains. Each repeat region of the *cagA* protein contains Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs, including a tyrosine phosphorylation site. It has now become more common to name the first repeat region as EPIYA-A and EPIYA-B segments and to name the second repeat region as EPIYA-C or EPIYA-D segments [22]. Western-type *cagA* contains EPIYA-A, EPIYA-B, and EPIYA-C segments. By contrast, East-Asian-type *cagA* contains the EPIYA-A, EPIYA-B, and EPIYA-D segments but not the EPIYA-C segment [4] (Fig. 6.2). Individuals infected with East Asian *cagA*-positive strains have higher degrees of inflammation, gastritis activity, and atrophy resulting in an increased risk of peptic ulcer or gastric cancer compared to patients infected with *cagA*-negative or Western *cagA*-positive strains [4, 11, 23, 24]. The genetic variation of *H. pylori* strains and the varied incidence of gastric cancer among populations might be explained at least in part by the structural polymorphism of *cagA* proteins among *H. pylori* strains circulating in different geographical areas [24].

However, there are limitations to explain the higher incidence of gastric cancer in East Asia using the concept of East-Asian-type *cagA* and Western-type *cagA* because the incidence of gastric cancer is also high in some regions where Western-type *cagA* strains are reported to account for the majority of *H. pylori* strains (e.g., in Peru and Columbia (age-standardized rate [ASR] per 100,000 population 21.2 and 17.4, respectively)) [25]. In addition, in Africa, the rate of *H. pylori* infection is high (e.g., 70–97% of patients with dyspepsia are infected with *H. pylori*, as are 80% of asymptomatic volunteers), but gastric cancer is generally uncommon; this seemingly contradictory situation is known as the "African enigma" [26]. The incidence of gastric cancer is extremely high in Mali, West Africa (ASR per 100,000 population 20.3), and the frequency of gastric cancer among women in this country is higher than it is among women in Japan (ASR per 100,000 population 19.3 vs. 18.2) [4]. Accordingly, these facts cannot be explained by the presence of East-Asian-type *cagA* versus Western-type *cagA* alone. In a study comparing the *cagA* gene repeat sequences found in Columbia (ASR per 100,000 population 17.4) with those found in the USA (ASR per 100,000 population 4.1) to explain the geographic difference in the incidence of gastric cancer, 100 *H. pylori* isolates from patients with simple gastritis (30 from Columbia and 70 from the USA) were analyzed; 57% of the isolates from Columbia had two EPIYA-C segments, whereas only 4% of the

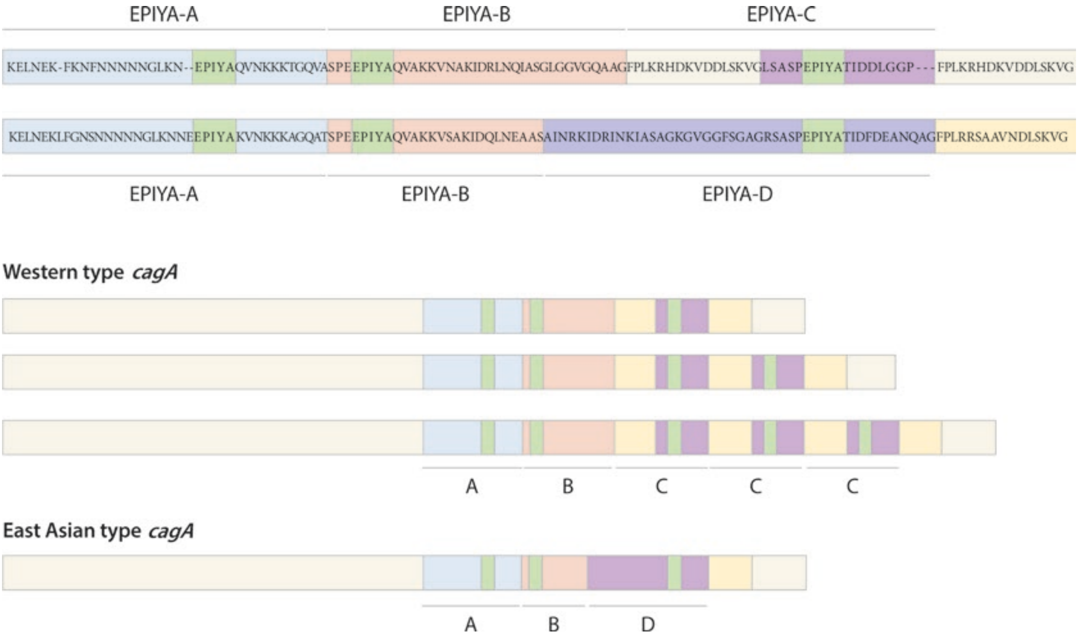
EPIYA-A
EPIYA-B
EPIYA-C
KELNEK-FKNFNNNNNGLKN--EPIYAQVNKKKTGQVASPEEPIYAQVAKKVNAKIDRLNQIASGLGGVGQAAGFPLKRHDKVDDLSKVGLSASPEPIYATIDDLGGP---FPLKRHDKVDDLSKVG
KELNEKLFGNSNNNNNGLKNNEEPIYAKVNKKKAGQATSPEEPIYAQVAKKVSAKIDQLNEAASAINRKIDRINKIASAGKGVGGFSGAGRSASPEPIYATIDFDEANQAGFPLRRSAAVNDLSKVG
EPIYA-A
EPIYA-B
EPIYA-D
Western type *cagA*
A
B
C
C
C
East Asian type *cagA*
A
B
D

Fig. 6.2 Structural polymorphism in *cagA*. Western-type *cagA* contains EPIYA-A, EPIYA-B, and EPIYA-C segments. By contrast, East-Asian-type *cagA* contains the EPIYA-A, EPIYA-B, and EPIYA-D segments but not the EPIYA-C segment. The EPIYA motif in each segment (shown in *green*) represents the tyrosine phosphorylation sites of *cagA*. The sequence flanking the tyrosine phosphorylation site of the EPIYA-D segment (EPIYATIDF), but not the EPIYA-C segment (EPIYATIDD), matches perfectly the consensus high-affinity binding sequence for the SH2 domains of SHP2. In Western countries, the incidence of gastric cancer is significantly higher in patients infected with strains containing multiple EPIYA-C segments than in patients infected with strains containing a single EPIYA-C segment (i.e., ABCCC vs. ABC). By contrast, almost all East-Asian strains contain a single EPIYA-D segment. *cagA* forms dimers in cells in a phosphorylation-independent manner, and the CagA multimerization (CM) sequence (also named the conserved repeat responsible for phosphorylation-independent activity [CRPIA] or MARK2/PAR1b kinase inhibitor [MKI]) in *yellow* was identified as the site responsible for dimerization, inhibition of MARK2/PAR1b kinase, and the interaction of *cagA* with activated c-Met (Adapted from Yamaoka [4])

isolates from the USA had two EPIYA-C segments (Y. Yamaoka, unpublished data) [4, 11]. Overall, the number of EPIYA-C segments may explain, to some extent, the geographic difference in the incidence of gastric cancer in Western countries [4]. Further research is required to determine whether these subtypes are involved in the pathogenesis of gastric cancer.

In the meta-analysis of case–control studies with age- and sex-matched controls, which provided raw data in East-Asian countries including Japan, Korea, and China, in eight studies, the pooled prevalence of *cagA* seropositivity was 71.6% (1019 out of 1423) in cases and 62.7% (1595 out of 2542) in controls. The estimated overall OR was 1.50 (95% confidence interval [CI], 1.30–1.72). In meta-analysis in a random effect model, the overall OR was 1.81 (95% CI, 1.30–2.11). This shows that the gastric cancer risk for *cagA*-positive cases was higher overall than in *H. pylori*-infected subjects; however, the OR in East-Asian countries was smaller than the result of the meta-analysis that included Western countries (1.81 vs. 2.64). In addition, the presence of anti-CagA antibodies increases the risk of gastric cancer in the *H. pylori*-negative population. The prevalence of anti-CagA antibodies ranged from 18.2 to 81.8% in gastric cancer patients and 9.8–60.2% in controls [27]. The lower frequency of higher titer IgG antibodies in advanced cancer may be due to the increasing extent of intestinal metaplasia associated with transition from the intestinal type of early gastric cancer to advanced cancer, such that the local environment is no longer ideal for the growth of *H. pylori* [4, 28]. CagA antibodies may be positive in patients who have a negative *H. pylori* serologic test since CagA antibodies can potentially remain positive for a longer period of time than the anti-*H. pylori* antibody [4, 27]. This evidence confirms that CagA antibodies can potentially remain positive for a longer period of time than the anti-*H. pylori* antibody [4]. Accordingly, anti-CagA antibody was related to gastric cancer in both *H. pylori*-positive and *H. pylori*-negative populations in the East-Asian population [4]. However, it is necessary to evaluate the availability of anti-*H. pylori* antibody plus anti-CagA antibody for screening for risk of gastric cancer.

6.3 Vacuolating Cytotoxin (*vacA*)

All the *H. pylori* strains have a functional *vacA*, which encodes a vacuolating cytotoxin. However, there is significant sequence diversity in *vacA* genes across the many *H. pylori* isolate strains [24, 29–33] (Fig. 6.3). There is variation in the vacuolating activity of different *H. pylori* strains, primarily due to differences in the *vacA* gene structure at the signal (s)-region (s1 and s2) and the middle (m)-region (m1 and m2) [4, 29]. In vitro experiments demonstrated that s1/m1 strains are the most cytotoxic, followed by s1/m2 strains, whereas s2/m2 strains have no cytotoxic activity and s2/m1 strains are rare [29]. There have been many reports that individuals infected with s1 or m1 *H. pylori* strains have an increased risk of peptic ulcer or gastric cancer compared with individuals infected with s2 or m2 strains [29–31]. Apart from the diversity regions “s,” “m,” and “i,” *vacA* possess “c” and “d” regions, but their role in the pathogenesis is still poorly understood. The c1 and d1 genotypes can be used as biomarkers as they are linked with the increased risk of gastric cancer. Moreover, the d1/c1 strains are associated with secreted vacuolating cytotoxin type encoding s1m1i1 and d2/c2 strains are linked with non-secreted vacuolating cytotoxin type encoding s2/m2/i2 genotypes [34, 35].

6.3.1 Geographic Differences in *vacA* Genotypes

It has been known that there are geographic differences in the distribution of both the *vacA* s- and m-region subtypes [29] (Fig. 6.4). Subtype s1a is predominant in Northern Europe and Australia, whereas subtype s1b is prevalent in South America. Subtype s1c is the major subtype in East Asia but is extremely rare in Western Europe. The most common *vacA* genotypes in Korea are s1c for the s-region and m1 for the m-region [29–31]. *vacA* s1/m1 is the most common strain in East Asia including Korea [30].

In East Asia including Korea, however, most *H. pylori* strains have an s1-type s-region; therefore, the pathogenic difference cannot be explained by the type of s region present [4].

The clear geographic differences in the distribution of both the *vacA* s- and m-region subtypes strongly suggest a geographic structure of *H. pylori* populations throughout the world [29]. The distribution of both s- and m-region allelic types in Central and South America is similar to that in the Iberian Peninsula. This distribution of *H. pylori vacA* genotypes may reflect the extensive cultural and socioeconomic relationships between these parts of the world during past centuries. Particular *H. pylori* strains may have been spread through Central and South America by colonization of these areas by Spanish and Portuguese descendants. Similarly, subtype s1a is more common in the northern European countries, and its predominance in Commonwealth countries (Canada and Australia) again may reflect historical relationships. It is unknown whether the evolution of both subtypes occurred in Europe. Within Europe, the distribution gradient of subtypes s1a (northern and eastern Europe) and s1b (Iberian Peninsula) was highly consistent among all tested strains from 12 different coun-

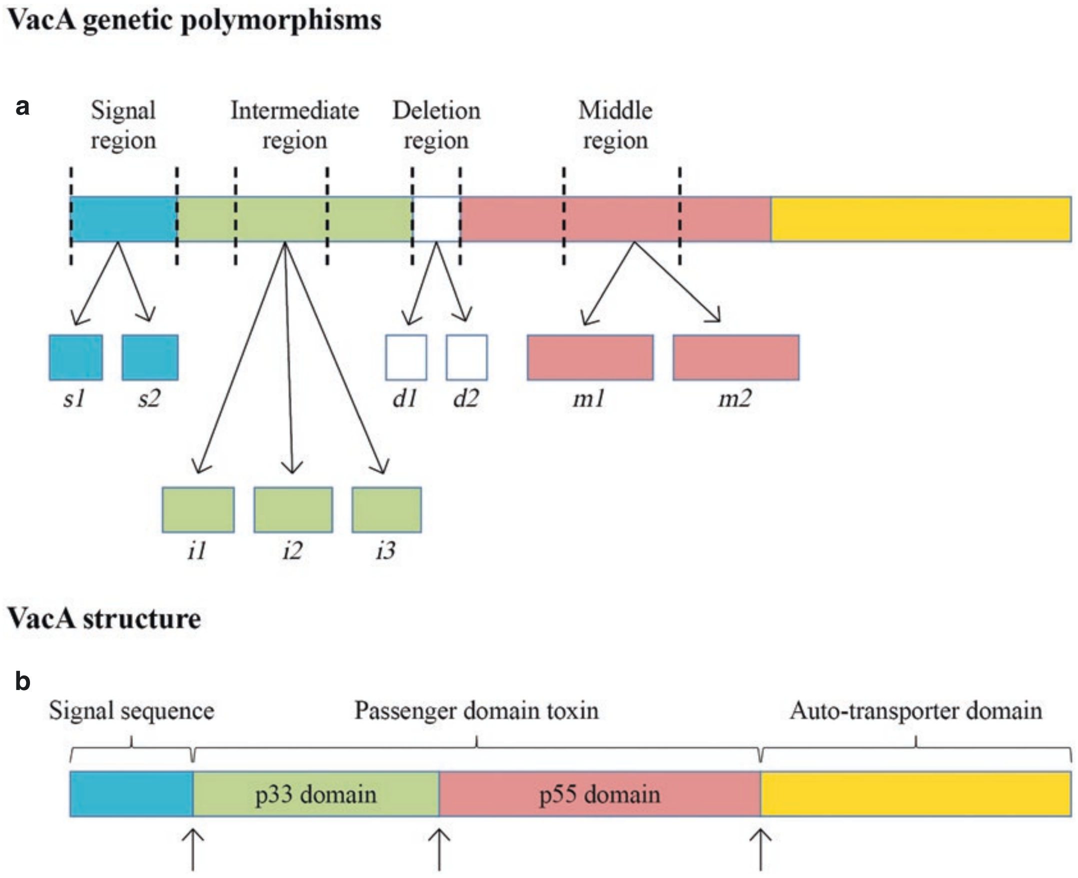
VacA genetic polymorphisms
a
Signal region
Intermediate region
Deletion region
Middle region
s1
s2
d1
d2
m1
m2
i1
i2
i3
VacA structure
b
Signal sequence
Passenger domain toxin
Auto-transporter domain
p33 domain
p55 domain

Fig. 6.3 *vacA* allelic diversity and structure. (**a**) *H. pylori* VacA allele polymorphisms, (**b**) VacA structure. The signal region allows for the passage of the pro-toxin across the inner-bacterial membrane. The passenger toxin domain consists of the N-terminal VacA fragment (p33) and the C-terminal VacA fragment (p55). The intermediate and middle regions are located in the p33 and p55 domains, respectively. dregion (d1 and d2) is classified based on the presence or absence of a segment ranging from about 60 to 100 nucleotides in length located at the junction of the p33 and p55 domains. The auto-transporter domain allows the toxin to translocate across the outer-bacterial membrane by forming a β-barrel. The p33 and p55 fragments may be cleaved from the β-barrel domain at some point during transit to, or in, the extracellular milieu to form the mature virulent subunits. Arrows indicate cleavage sites (Adapted from Hanafiah et al. [24])

tries. If subtypes s1a and s1b already existed for a long time in Europe and were freely movable, a more homogeneous distribution would have been expected. On the other hand, if transmission of *H. pylori* is highly local, only occurring over very short distances during childhood, this may have prevented a broader geographic distribution of each subtype. An alternative hypothesis would be that the distribution of different subtypes reflects a particular adaptation of *H. pylori* to specific host populations [29–31, 36, 37].

In the Korean *H. pylori* strain, a wide diversity has been observed in the *vacA* s1-region. In the study performed by Kim et al. [12], *vacA* s1a–s1c was determined as the most common subtype in South Korea, and considering the positivity of genotypes, *vacA* s1c and s1a were identified as the major genotypes. s1a–s1c was significantly more frequent in benign gastric ulcer (73.2%, $p = 0.023$), gastric cancer (73.6%, $p = 0.018$), and dysplasia (71.9%, $p = 0.048$) than control (58.7%) (Tables 6.2 and 6.3).

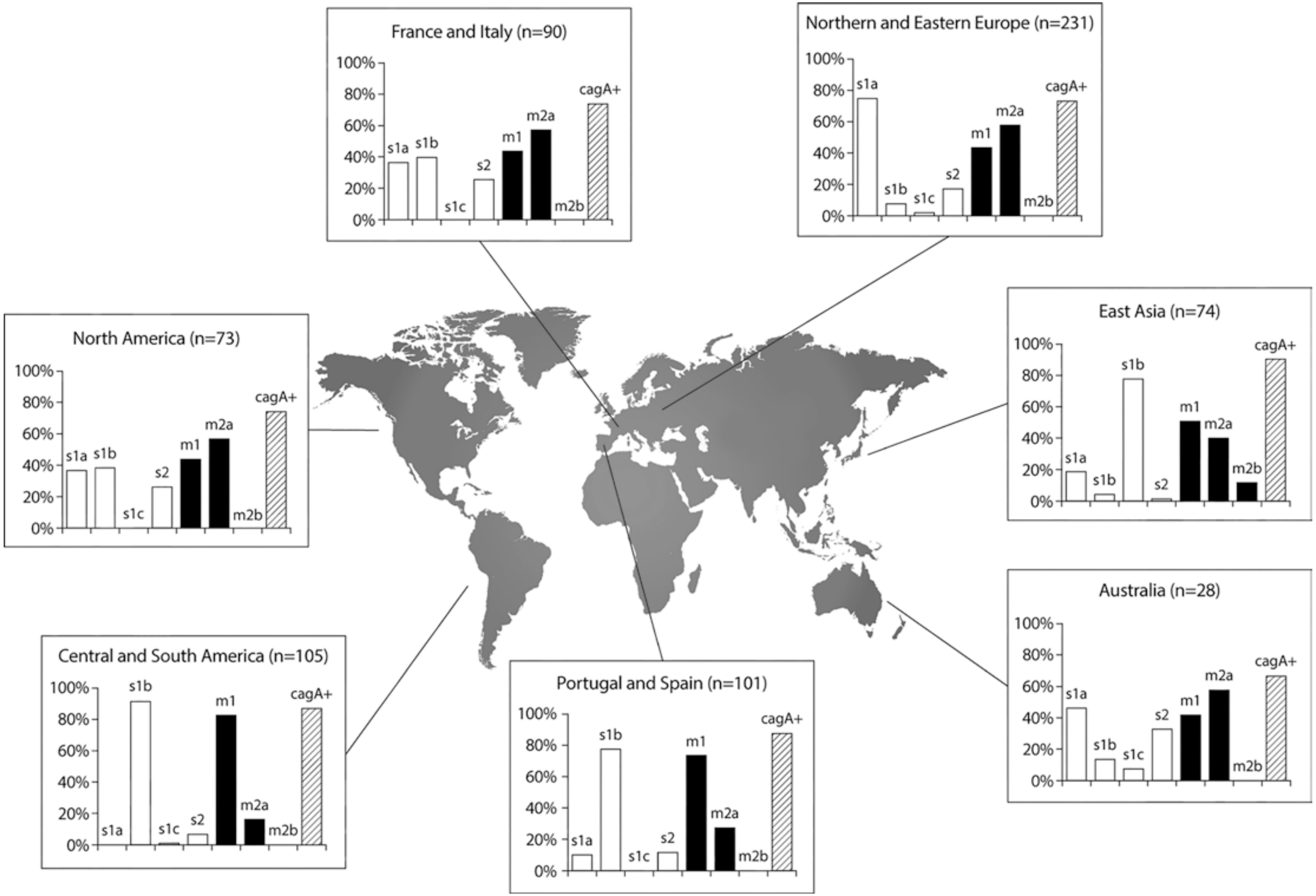

Fig. 6.4 Distribution of *vacA* s- and m-region and *cagA* genotypes of *H. pylori* strains from different parts of the world. For each region, the prevalence of each type (s1a, s1b, s1c, s2, m1, m2a, and m2b; *cagA* positive) is given as a percentage of the total number of strains (shown in *parentheses*). Only strains containing a single *vacA* genotype are represented (Adapted from Van Doorn et al. [29])

Table 6.2 Positivity of *vacA*, *cagA*, *iceA*, *oipA*, and *dupA* of *H. pylori* in 401 colonies from Korea

	Control ($n = 75^{a}$)	BGU ($n = 71^{a}$)	DU ($n = 102^{a}$)	Stomach cancer ($n = 121^{a}$)	Dysplasia ($n = 32^{a}$)	Total ($n = 401^{a}$)	p value
vacA s1 (%)	75/75 (100.0)	71/71 (100.0)	102/102 (100.0)	121/121 (100.0)	32/32 (100.0)	401/401 (100.0)	0.999
s1a	61/75 (81.3)	67/71 (94.4)^{b}	84/102 (82.4)	108/121 (89.3)	31/34 (96.9)^{b}	351/401 (87.5)	0.026
s1b	9/75 (12.0)	6/71 (8.5)	15/102 (14.7)	17/121 (14.0)	7/32 (21.9)	54/401 (13.5)	0.436
s1c	67/75 (89.3)	58/71 (81.7)	83/102 (81.4)	113/121 (93.4)	28/32 (87.5)	349/401 (87.0)	0.052
vacA m1	70/75 (93.3)	69/71 (97.2)	89/102 (87.3)	116/121 (95.9)	32/32 (100.0)	376/401 (92.9)	0.018
vacA m2	5/75 (6.7)	2/71 (2.8)	13/102 (12.7)	5/121 (4.1)	0/32 (0.0)	25/401 (6.2)	0.018
vacA i1	75/75 (100.0)	71/71 (100.0)	102/102 (100.0)	121/121 (100.0)	32/32 (100.0)	401/401 (100.0)	0.999
vacA i2	10/75 (13.3)	14/71 (19.7)	10/102 (9.8)	15/121 (12.4)	1/32 (3.1)	50/401 (12.5)	0.153
cagA	59/66 (89.4)	53/64 (82.8)	82/96 (85.4)	96/106 (90.6)	23/27 (85.2)	313/359 (87.2)	0.586
EPIYA-C	1/46 (2.2)	1/39 (2.6)	3/52 (5.8)	2/78 (2.6)	0/19 (0.0)	7/234 (3.0)	0.709
EPIYA-D	45/46 (97.8)	38/39 (97.4)	49/52 (94.2)	76/78 (97.4)	19/19 (100.0)	227/234 (97.0)	0.709
*iceA*1	74/75 (98.7)	68/71 (95.8)	96/102 (94.1)	114/121 (94.2)	32/32 (100.0)	384/401 (95.8)	0.359
*iceA*2	27/75 (36.0)	27/71 (38.0)	45/102 (44.1)	67/119 (56.3)^{b}	16/32 (50.0)	182/399 (45.6)	0.036
oipA	63/75 (84.0)	63/71 (88.7)	96/100 (96.0)^{b}	114/120 (95.0)^{b}	27/32 (84.4)	363/398 (91.2)	0.015
dupA	12/72 (16.7)	52/64 (81.3)^{b}	74/99 (74.7)^{b}	22/95 (23.2)	10/24 (41.7)^{b}	170/354 (48.0)	<0.001

Missing values are not included. Each number behind the dash is the total number of colonies which were analyzed

BGU benign gastric ulcer, *DU* duodenal ulcer, *H. pylori Helicobacter pylori*, *EPIYA-C* Western-type *cagA*, *EPIYA-D* East-Asian-type *cagA*

Adapted from Kim et al. [12]

[a] Total number of each group

[b] $p < 0.05$, compared with the control group

Table 6.3 *vacA* and *iceA* subtypes of *H. pylori* in 401 colonies from Korea

	Control (n = 75[a])	BGU (n = 71[a])	DU (n = 102[a])	Stomach cancer (n = 121[a])	Dysplasia (n = 32[a])	Total (n = 401[a])
vacA s1 (%)						
a	8/75 (10.7)	9/71 (12.7)	12/102 (11.8)	4/121 (3.3)	1/32 (3.1)	34/401 (8.5)
b	–	–	–	–	–	–
c	14/75 (18.7)	4/71 (5.6)[b]	18/102 (17.6)	11/121 (9.1)	1/32 (3.1)[b]	48/401 (12.0)
a–b	–	4/71 (5.6)	7/102 (6.9)	2/121 (1.7)	3/32 (9.4)	16/401 (4.0)
a–c	44/75 (58.7)	52/71 (73.2)[b]	57/102 (55.9)	89/121 (73.6)[b]	23/32 (71.9)[b]	265/401 (66.1)
Others	9/75 (12.0)	2/71 (2.8)	8/102 (7.8)	15/121 (12.4)	4/32 (12.5)	38/401 (9.5)
vacA m						
m1	70/75 (93.3)	69/71 (97.2)	89/102 (87.3)	116/121 (95.9)	32/32 (100.0)	376/401 (92.9)
m2	5/75 (6.7)	2/71 (2.8)	13/102 (12.7)	5/121 (4.1)	0/32 (0.0)	25/401 (6.2)
vacA i						
i1	65/75 (86.7)	57/71 (80.3)	92/102 (90.2)	106/121 (87.6)	31/32 (96.9)	351/401 (87.5)
i2	–	–	–	–	–	–
i1 and i2	10/75 (13.3)	14/71 (19.7)	10/102 (9.8)	15/121 (12.4)	1/32 (3.1)	50/401 (12.5)
vacA						
s1i1m1	60/75 (80.0)	56/71 (78.9)	83/102 (81.4)	103/121 (85.1)[b]	31/32 (96.9)[b]	333/401 (83.0)
s1i1m2	5/75 (6.7)	1/71 (1.4)	9/102 (8.8)	3/121 (2.5)	–	18/401 (4.5)
s1i1 and i2m1	10/75 (13.3)	13/71 (18.3)	6/102 (5.9)	13/121 (10.7)	1/32 (3.1)	43/401 (10.7)
s1i1 and i2m2	–	1/71 (1.4)	4/102 (3.9)	2/121 (1.7)	–	7/401 (1.7)
iceA						
1	48/75 (64.0)	44/71 (62.0)	57/102 (55.9)	51/119 (42.9)[b]	16/32 (50.0)	216/399 (54.1)
2		3/71 (4.2)	6/102 (5.9)	7/119 (5.9)	–	16/399 (4.0)
1 and 2	27/75 (36.0)	24/71 (33.8)	39/102 (38.2)	61/119 (51.3)[b]	16/30 (50.0)	167/399 (41.9)

Missing values are not included. Each number behind the dash is the total number of colonies that were analyzed

BGU benign gastric ulcer, *DU* duodenal ulcer, *H. pylori Helicobacter pylori*

Adapted from Kim et al. [12]

[a] Total number of each group

[b] $p < 0.05$, compared with the control group

On the other hand, a study performed in Korea for peptic ulcer and gastritis patients by Park et al. [36] showed that s1c, s1a, and s1b were found in 66.1%, 35.6%, and 0% of *H. pylori* isolates, respectively. Another study [9] analyzed *H. pylori* isolates from Seoul, Korea; s1c and s1a were found in 90% and 7%, respectively. In both studies, s1 subtypes were not related to clinical outcomes. This explanation may support the reason for the possible infection of multiple strains in Korea and other countries. However, further studies will be required to clarify these diversities.

Regarding the combination of the *vacA* s and m genotypes, the Japanese strain from Okinawa showed that the *vacA* s1/m1 genotype was significantly higher in strains from gastric ulcer (79.2%) and gastric cancer (87.5%) than those from gastritis (59.2%) (p = 0.002 and 0.006, respectively). The prevalence of the *vacA* s1/m2 genotype tended to be higher in strains from patients with duodenal ulcer than those from patients with gastritis (27.2% vs. 17.3%), although the difference did not reach statistical significance (p = 0.08). The prevalence of the *vacA* s2/m2 genotype was significantly higher in strains from gastritis patients than in those from gastric ulcer, duodenal ulcer, and gastric cancer patients (22.4% vs. 11.9%, 10.5%, and 4.2%, p = 0.04, 0.01, and 0.04, respectively) [38–40]. These results suggested that diverse *vacA* genotypes contribute to the clinical outcomes in Okinawa and the low incidence of gastric cancer in Okinawa [4, 11].

With respect to the m-region, there is variation within East Asia. Although m1 strains are common in parts of north East Asia, such as Japan and South Korea, m2 strains are predominant in parts of south East Asia, such as Taiwan and Vietnam [40–42]. As the incidence of gastric cancer is higher in the north than in the south of East Asia, the m-region may play a role in the regional difference in disease pattern [4]. Even within Vietnam, the incidence of gastric cancer is approximately 1.5 times higher in Hanoi in the north than in Ho Chi Minh in the south of the country. A comparison of two geographically distant cities in Vietnam, Hanoi, and Ho Chi Minh showed that the *vacA* m1 genotype, thought to be more toxic than the *vacA* m2 type, is more prevalent in Hanoi, where the incidence of gastric cancer is higher than in Ho Chi Minh [41, 42]. These data support the hypothesis that the *vacA* m1 type is closely associated with gastric carcinogenesis [4].

In 2007, a third disease-related region of *vacA* was identified between the s-region and the m-region; it was named the intermediate (i)-region [43]. Yamaoka et al. [4] reported that all s1/m1 strains were classified as type i1, and all s2/m2 strains were classified as type i2, but s1/m2 strains were classified as either type i1 or i2, and i1 strains were shown to be more pathogenic. Typing of the i-region was also reported to be more effective for determining the risk of gastric cancer in Iranian patients than typing of the s-region or m-region [44]. However, in a study of patients from East and Southeast Asia, there was no association between the i-region and disease [11, 45].

More recently, a fourth disease-related region—the deletion (d) region—was identified between the i-region and the m-region [4]. The d-region is divided into d1 (no deletion) and d2 (a 69–81 bp deletion). The study of Western strains demonstrated that d1 was a risk factor for gastric mucosal atrophy; however, almost all East-Asian strains are classified as s1/i1/d1. Therefore, further researches are needed to clarify the association between d- or i- region and clinical outcome [11, 44, 45].

The role of *H. pylori* virulence factors in the eradication rate has been deeply challenged. A recent meta-analysis showed that infection with *cagA*-positive strains of *H. pylori* significantly increases the risk of metronidazole resistance in Western countries. In addition, *vacA* s1m1 increases resistance to amoxicillin and levofloxacin in Western countries. The *vacA* s1m1 significantly increases resistance to metronidazole, while the *vacA* s1m2 decreases resistance to clarithromycin and metronidazole. Additionally, antibiotic resistance to clarithromycin, metronidazole, amoxicillin, tetracycline, and levofloxacin in less virulent *H. pylori* strains (carrying *vacA* s2m2 genotype) is significantly lower than others [46]. More studies are warranted to clarify the relationship between virulent factors of *H. pylori* and antibiotic resistance.

6.4 Induced by Contact with Epithelium (*iceA*)

An initial series of studies showed that *iceA* has two main allelic variants, *iceA*1 and *iceA*2. The expression of *iceA*1 was upregulated on contact between *H. pylori* and human epithelial cells, and the *iceA*1 genotype was linked with enhanced mucosal IL-8 expression and acute antral inflammation [4, 11, 47]. The *iceA* type 1 allele is reported to be predominant in Japan and Korea, and the *iceA* type 2 allele is reported to be predominant in the United States and Colombia [4, 9, 40, 47].

In a meta-analysis [48] including 50 studies with a total of 5357 patients to confirm the relationship between the *iceA* allelic type and clinical outcomes, the overall prevalence of *iceA*1 was significantly higher by 64.6% (1791/2771) in Asian countries than in Western countries (64.6% vs. 42.1%), whereas the prevalence of *iceA2* was more prevalent in Western countries than in Asian countries (45.1% vs. 25.8%). Sensitivity analysis showed that the presence of *iceA1* was significantly associated with peptic ulcer (OR 1.25; 95% CI, 1.08–1.44); however, the presence of *iceA2* was inversely associated with peptic ulcer (OR 0.76; 95% CI, 0.65–0.89). These findings were significant in Western countries. In addition, the presence of *iceA* was not associated with gastric cancer. Most studies examined the *cagA* status; however, only 15 studies examined the correlation and only 2 showed a positive correlation between the presence of *cagA* and *iceA1*. It is possible that *iceA* is a discriminating factor for peptic ulcer, which is independent of *cagA* [4, 11]. However, it is a result that has not been confirmed in other countries, such as Japan and Korea [9, 11, 47]. Kim et al. [12] reported that the positivity of *iceA*1 in Korean *H. pylori* isolates was more than 95%, and *iceA*2 was variable from 35 to 55% among clinical diseases. In addition, strains expressed with *iceA*2 alone were only about 5%, and most strains were detected with *iceA*1 along with *iceA*2. This result could support a wide diversity of *H. pylori* infections in South Korea.

In summary, despite numerous attempts to relate *vacA* genotypes to outcome or disease pathogenesis, no consistent associations or demonstrable biologic basis for the putative associations has appeared. Further studies are warranted.

6.5 Outer Membrane Protein

Outer membrane protein has been shown to act as an adhesion that facilitates bacterial attachment to the host epithelium. Approximately, 4% of the *H. pylori* genome is predicted to encode outer membrane proteins. There have been many studies that investigate the expression status of outer membrane proteins such as OipA, BabA, or BabB in different clinical outcomes [4, 49–51].

6.5.1 Outer Inflammation Protein (*oipA*)

oipA was initially identified as a proinflammatory response-inducing protein based, in part, on the fact that *oipA* isogenic mutants reduced the production of IL-8 from gastric epithelial cell lines [4, 49–51]. Transcription of *IL-8* genes in both *oipA* and *cag* PAI dependent through interactions with different binding sites are involved, such as transcription factors within the IL-8 promoter for nuclear factor-κB (NF-κB), activator protein 1 (AP-1), and interferon-stimulated responsive element (ISRE)-like element [4]. *oipA* functional status was related to clinical presentation, *H. pylori* density, and gastric inflammation. *cag* PAI, *babA2*, or *vacA* status appears important only as surrogate markers for a functional *oipA* gene. It is also important to reconfirm that the presence of *cagA*, *vacA*, and *oipA* are linked such that typically *H. pylori* either produces all of these proteins or none of them and that clinical outcomes, such as peptic ulcer and gastric cancer, are associated with strains with and without these virulence factors. However, strains with recognized virulence factors tend to produce more severe inflammation and are associated with higher risk of these important clinical outcomes [4, 11].

A meta-analysis reported that the oipA “on,” but not “off,” status is significantly associated with an increased risk of peptic ulcer disease (OR 3.97) and gastric cancer (OR 2.43), especially in Western countries [52]. However, strains in Asia appeared to be different from those in Western countries in the aspect of outer membrane proteins and their actions [4, 11]. Kim et al. [12] reported that *oipA* was more frequently detected in duodenal ulcers and gastric cancer, but a significant effect on gastroduodenal diseases was not found in Korean *H. pylori* isolates. These results could also be evidence of the different distribution of virulence factors according to geographic differences.

Polymorphism of *oipA* is driven by changes in *oipA* copy number. Two copies of *oipA* were identified in a subset of strains that were part of a collection of clinical isolates from Japanese, Korean, and Amerindian populations. Conversely, this genotype was not seen in European and West African strains [53, 54]. A recent study [55] conducted a large-scale molecular epidemiologic analysis using *H. pylori* clinical isolates obtained from two geographically distinct populations showed that most Korean isolates (98.7%) possessed two copies of *oipA* located at two specific loci (A and B) while all US isolates contained only one copy of *oipA* at locus A. Furthermore, most Korean *oipA* (94.8%) possessed three or less CT repeats while most US *oipA* (96.6%) contained five or more CT repeats. Among the two copies, all Korean *H. pylori* possessed at least one *oipA* "on" phase variant while the single copy of *oip*A in US isolates showed 56.2% "on" and 43.8% "off." These findings suggest that diversity in the *oipA* gene copy number, CT repeats, and phase variation among *H. pylori* from different populations may confer a benefit in adaptation to particular host populations [55].

The *H. pylori oipA* has been demonstrated to be a potential antigen for a vaccine. Recently, *oipA* has been tested in mice and vector-based approaches, and/or multicomponent vaccines have been investigated [56]. The study showed that the *H. pylori oipA* encoding construct is capable of inducing humoral and cellular responses in immunized mice. The antibody response profiles elicited by the DNA vaccine alone administered intradermally (the gene gun method) showed that it produced a Th2 immune response, while co-delivery of *IL-2* and *LTB* gene encoding constructs promoted a Th1-biased immune response. Mahboubi et al. [57] inoculated mice with the OipA vaccine, and the amount of *H. pylori* colonized in the stomach was significantly reduced, as well as the inflammation after some time. Therefore, immunogenic OipA could be used as an *H. pylori* vaccine to make up for the increasing antibiotic resistance of *H. pylori*. Further studies warranted for developing vaccination for *H. pylori*.

6.5.2 Duodenal Ulcer Promoting Gene A (*dupA*)

In 2005, the first disease-specific *H. pylori* virulence factor that induced duodenal ulcer and had a suppressive action on gastric cancer was identified and was named duodenal ulcer promoting gene A (*dupA*) [58, 59]. The presence of *dupA* was associated with elevated IL-8 production in the antrum (i.e., antrum-predominant gastritis—a feature of duodenal ulcer disease) and has been reported to induce IL-12 production from monocytes [60].

In an initial study of a total of 500 *H. pylori* isolates, including 160 from Japan, 175 from South Korea, and 165 from Colombia, the positive rate for the *dupA* gene was high in patients with duodenal ulcer and low in patients with gastric cancer, regardless of a patients' nationality (42% vs. 9% on average) [58]. In the study analyzed 401 Korean *H. pylori* isolates by Kim et al. [12], the prevalence of *dupA* was 48.0%. Infection by *dupA*-positive *H. pylori* showed an increased risk of gastric ulcer (OR 33.06; 95% CI, 11.91–91.79) and duodenal ulcer (OR 15.60; 95% CI, 6.49–37.49). More than 75% of colonies with gastric ulcer and duodenal ulcer expressed *dupA*, which suggests that *dupA* may be a fundamental factor for the development of peptic ulcer diseases in South Korea.

However, Brazil, Singapore, Malaysia, and Japan failed to demonstrate a correlation between the presence of the *dupA* gene and disease [4]. An academic report on Brazilian strains by Queiroz et al. [61] showed that a *dupA* gene mutation (deletion or insertion) was found in 50% of patients with gastric cancer, whereas it was found in only approximately 20% of patients with duodenal ulcer. As a result, the positive rate for the functional *dupA* gene was considerably higher in patients with duodenal ulcer than in patients with gastric cancer. The observed discrepancy of *dupA* link with disease outcomes might be associated with the plasticity region of *H. pylori* or the limitation of PCR to detect the various forms of *dupA* gene [62]. Further investigation might clarify the effect of functional *dupA* on various gastroduodenal diseases.

6.5.3 Blood Group A Antigen-Binding Adhesion (*babA*)

BabA is an important protein of outer membrane protein (OMP) family with an approximate molecular weight of around 75–80 kDa. Two closely related paralogs, BabB and BabC, have been identified. Although the BabA-mediated attachment provides survival adaptation and persistent infections, the functions of BabB and BabC are not yet known [63–67] (Fig. 6.5).

Interactions between BabA and Lewis b (Leb)-related antigens are the best characterized adhesion receptor interactions in *H. pylori*. The *babA* gene was initially cloned from strain CCUG17875, which contains a silent *babA*1 gene and an expressed *babA*2 gene [4, 64]. A number of studies have suggested a relationship between *babA2*-positive *H. pylori* and increased cellular mucosal inflammations as well as an increased risk of developing clinical outcomes [4].

In Western countries, infection with BabA producing strains has been associated with an increased risk for peptic ulcer disease development [64]. A recent study highlighted the adherence of strains with a high avidity mediated by BabA-positive pediatric ulcerogenic *H. pylori* strains [68]. However, Kim et al. [66] reported that the incidence of *babA* was low and was not related to peptic ulcer disease in Korea. The presence of the *babA* genes in *H. pylori* isolates from peptic ulcer and gastritis patients was 27.3% and 26.3%, respectively ($p = 0.578$). In addition, the four pathogenicity-related genes, *cagA*, *vacA* s1c/m1, *iceA1*, and *babA*, did not correlate with other genes. In a Japanese study by Fujimoto et al. [69], all strains from East Asia expressed BabA protein, and 24 (9.8%) of the Western strains were *babA* negative. For these strains, the *babA*-negative status was correlated inversely with *cagA* or *vacA* s status (i.e., only 1 [4.2%] and none [0%] of these *babA*-negative strains were *cagA*- or *vacA* s1-positive, respectively). Most (91%) Western strains were classified as either *cagA*-positive/*vacA* s1-positive/BabA-H (triple positive, 76%), *cagA*-positive/*vacA* s1-positive/BabA-L (6.1%), or *cagA*-negative/*vacA* s2-positive/*babA*-negative strains (9.4%). However, there was no relationship between the triple-positive strains and clinical outcome. *babA*-negative status is associated with mild gastric injury and lower *H. pylori* density. *babA*-negative strains are also associated infrequently with duodenal ulcer or gastric cancer. Because *babA*-negative status is linked closely to *cagA*-negative/*vacA* s2 status, potential interactions between these different putative virulence factors cannot be ruled out.

Based on the binding preferences with ABO blood group antigens, the *BabA*-positive *H. pylori strains* can be of two types. The "specialists" prefer to bind only blood group O-specific glycans, while the "generalists" bind blood group O and blood group A and B-specific glycans [70]. The capability of both specialist and generalist

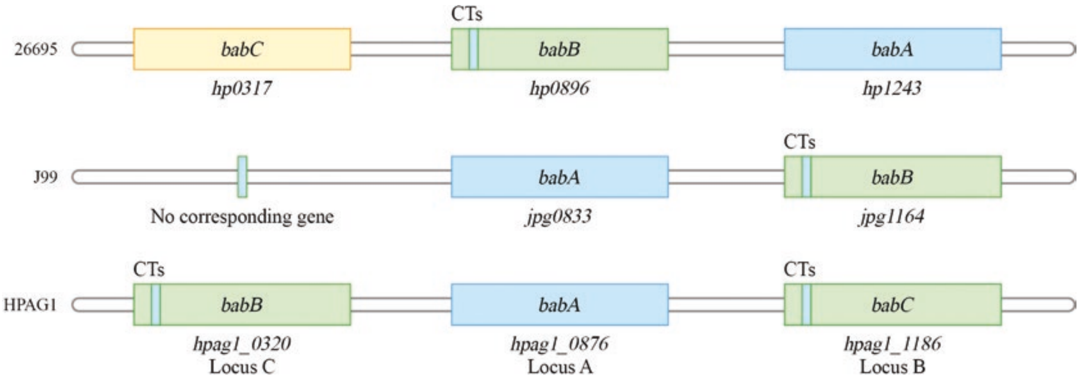

Fig. 6.5 Genomic locations of the *babA*, *babB*, and *babC* genes in strains J99, 26695, and HPAG1. *CTs* CT dinucleotide repeats (Adapted from Chen et al. [67])

strains to bind with blood group O-specific glycans explains why people with blood group O are at a high risk for developing duodenal ulcer diseases [70].

In summary, it remains unclear how BabA expression is regulated or if expressing low levels of BabA has a direct role in the pathogenesis of duodenal ulcer or gastric cancer [11]. Further studies are warranted.

6.5.4 HomA and HomB

The *hom* genes are known as a small paralogous family of adhesion proteins and are distinguished from other OMPs by the signal sequence and hydrophobic motif located in the C-terminal domain [71, 72] The *hom* family consists of four classes *homA*, *homB*, *homC*, and *homD* so that *homA* and *homB* are encoded by one locus, while each of *homC* and *homD* is encoded by a distinct locus [73]. Despite the 90% similarity between *homA* and *homB*, studies show that the distribution of each is specific in each geographic area, so that *homA* is a diagnostic marker for East Asian strains, and *homB* has a global distribution [73, 74].

The *homB* gene enhances the attachment of *H. pylori* to the gastric epithelium, leading to dysregulation of normal signaling pathways and genetic instability [75, 76]. In addition, this gene increases the risk of gastric cancer through interferences such as inducing the inflammatory response, persistent infection, and gastric atrophy [77].

Recent meta-analysis showed that the *homB*-positive strains significantly increase the risk of peptic ulcer (OR 1.36; 95% CI, 1.07–1.72), especially in western countries (OR 1.61; 95% CI, 1.20–2.1). Moreover, a positive association between the *homB* gene and the risk of gastric cancer (OR 2.16; 95% CI, 1.37–3.40) was observed. In addition, based on subgroup analysis, it was found that the presence of this gene in *H. pylori* strains increases the risk of gastric cancer in the Asian population (OR 3.71; 95% CI, 1.85–7.45), suggesting the presence of the *homB* gene in *H. pylori* strains contributes to the development of primary infection to severe clinical outcomes [71].

6.6 Conclusion

The *H. pylori* virulence factors are important for clarifying the role of *H. pylori* in the regional differences in gastric cancer distribution and the pathogenesis of clinically significant diseases such as gastric cancer or peptic ulcer. If all the studies to data are put together, *cagA*, *vacA*, *oipA,* and *babA* are the factors associated with gastric cancer, and *dupA* can be considered to be an important virulent factor for duodenal ulcer, but because different studies have shown different results and particularly results were different by geographic region, more research is needed. The polymorphisms in host genes encoding the immune effector proteins play important roles in the inter-individual variations in clinical outcomes since they directly add to the susceptibility of an individual to *H. pylori* infection and related diseases.

The biggest reasons why there is a limit in clarifying the relationship between *H. pylori* and diseases only by the virulence factors are as follows: *H. pylori* is composed of nearly 1600 genes; thus, it is possible that a pathogenic gene, which has not yet been identified, plays a critical role. Moreover, the pathogenesis of gastroduodenal diseases including gastric cancer involves several factors such as diet, environmental changes caused by human movement, host factors, or duration of *H. pylori* infection, which should be taken into account along with the virulent factors in the pathogenesis of diseases. In addition, it is needed to better understand and interpret the research methods and terminology when the study results associated with *H. pylori* virulence factors are comprehended and analyzed. Tests for virulence factors are to be made through the use of the strains isolated from the host in vitro or an animal model; however, the actual results caused by these virulence factors in the human body can be different from the results obtained in the laboratory. Therefore, it should be kept in mind that the actual working mechanisms of the *H. pylori* virulence factors in the host can be much more complicated and diverse than we imagine.

References

1. Moodley Y, Linz B, Bond RP, Nieuwoudt M, Soodyall H, Schlebusch CM, et al. Age of the association between *Helicobacter pylori* and man. PLoS Pathog. 2012;8:e1002693.
2. Peek RM Jr, Blaser MJ. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. Nat Rev Cancer. 2002;2:28–37.
3. Linz B, Balloux F, Moodley Y, Manica A, Liu H, Roumagnac P, et al. An African origin for the intimate association between humans and *Helicobacter pylori*. Nature. 2007;445:915–8.
4. Yamaoka Y. Mechanisms of disease: *Helicobacter pylori* virulence factors. Nat Rev Gastroenterol Hepatol. 2010;7:629–41.
5. Devi SM, Ahmed I, Khan AA, Rahman SA, Alvi A, Sechi LA, et al. Genomes of *Helicobacter pylori* from native Peruvians suggest admixture of ancestral and modern lineages and reveal a western type cag-pathogenicity island. BMC Genomics. 2006;7:191.
6. Yamaoka Y. *Helicobacter pylori* typing as a tool for tracking human migration. Clin Microbiol Infect. 2009;15:829–34.
7. Ching CK, Wong BC, Kwok E, Ong L, Covacci A, Lam SK. Prevalence of *CagA*-bearing *Helicobacter pylori* strains detected by the anti-*CagA* assay in patients with peptic ulcer disease and in controls. Am J Gastroenterol. 1996;91:946–53.
8. Weel JF, van der Hulst RW, Gerrits Y, Roorda P, Feller M, Dankert J, et al. The interrelationship between cytotoxin-associated gene A, vacuolating cytotoxin, and *Helicobacter pylori*-related diseases. J Infect Dis. 1996;173:1171–5.
9. Yamaoka Y, Kodama T, Gutierrez O, Kim JG, Kashima K, Graham DY. Relationship between *Helicobacter pylori iceA*, *cagA*, *and vacA* status and clinical outcome: studies in four different countries. J Clin Microbiol. 1999;37:2274–9.
10. Crabtree JE, Taylor JD, Wyatt JI, Heatley RV, Shallcross TM, Tompkins DS, et al. Mucosal IgA recognition of *Helicobacter pylori* 120 kDa protein, peptic ulceration and gastric pathology. Lancet. 1991;338:332–5.
11. Shiota S, Suzuki R, Yamaoka Y. The significance of virulence factors in *Helicobacter pylori*. J Dig Dis. 2013;14:341–9.
12. Kim JY, Kim N, Nam RH, Suh JH, Chang H, Lee JW, et al. Association of polymorphisms in virulence factor of *Helicobacter pylori* and gastroduodenal diseases in South Korea. J Gastroenterol Hepatol. 2014;29:984–91.
13. Kim YS, Kim N, Kim JM, Kim MS, Park JH, Lee MK, et al. *Helicobacter pylori* genotyping findings from multiple cultured isolates and mucosal biopsy specimens: strain diversities of *Helicobacter pylori* isolates in individual hosts. Eur J Gastroenterol Hepatol. 2009;21:522–8.
14. Allison CC, Ferrero RL. Role of virulence factors and host cell signaling in the recognition of *Helicobacter pylori* and the generation of immune responses. Future Microbiol. 2010;5:1233–55.
15. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, et al. *Cag*, a pathogenicity island of *Helicobacter pylori*, encodes type 1-specific disease-associated virulence factors. Proc Natl Acad Sci U S A. 1996;93:14648–53.
16. Crabtree JE, Covacci A, Farmery SM, Xiang Z, Tompkins DS, Perry S, et al. *Helicobacter pylori* induced interleukin-8 expression in gastric epithelial cells is associated with *CagA* positive phenotype. J Clin Pathol. 1995;48:41–5.
17. Sharma SA, Tummuru MKR, Miller GG, Blaser MJ. Interleukin-8 response of gastric epithelial cell lines to *Helicobacter pylori* stimulation in vitro. Infect Immun. 1995;63:1681–7.
18. Cover TL, Glupczynski Y, Lage AP, Burette A, Tummuru MK, Perez-Perez GI, et al. Serologic detection of infection with *CagA*$^+$ *Helicobacter pylori* strains. J Clin Microbiol. 1995;33:1496–500.
19. Crabtree JE, Wyatt JI, Sobala GM, Miller G, Tompkins DS, Primrose JN, et al. Systemic and mucosal response to *Helicobacter pylori* in gastric mucosa. Gut. 1993;34:1339–43.
20. Kuipers EJ, Pérez-Pérez GI, Meuwissen SG, Blaser MJ. *Helicobacter pylori* and atrophic gastritis: importance of the *CagA* status. J Natl Cancer Inst. 1995;87:1777–80.
21. Keikha M, Sahebkar A, Yamaoka Y, Karbalaei M. Helicobacter pylori cagA status and gastric mucosa-associated lymphoid tissue lymphoma: a systematic review and meta-analysis. J Health Popul Nutr. 2022;4:2.
22. Hatakeyama M. Oncogenic mechanisms of the *Helicobacter pylori CagA* protein. Nat Rev Cancer. 2004;4:688–94.
23. Matsunari O, Miftahussurur M, Shiota S, Suzuki R, Vilaichone R, Uchida T, et al. Rare Helicobacter pylori virulence genotypes in Bhutan. Sci Rep. 2016;6:22584.
24. Hanafiah A, Lopes BS. Genetic diversity and virulence characteristics of Helicobacter pylori isolates in different human ethnic groups. Infect Genet Evol. 2020;78:104135.
25. Kersulyte D, Mukhopadhyay AK, Velapatiño B, Su W, Pan Z, Garcia C, et al. Differences in genotypes of *Helicobacter pylori* from different human populations. J Bacteriol. 2000;182:3210–8.
26. Holcombe C. *Helicobacter pylori*: the African enigma. Gut. 1992;33:429–31.

27. Shiota S, Matsunari O, Watada M, Yamaoka Y. Serum *Helicobacter pylori CagA* antibody as a biomarker for gastric cancer in east-Asian countries. Future Microbiol. 2010;5:1885–93.
28. Asaka M, Kimura T, Kato M, Kudo M, Miki K, Ogoshi K, et al. Possible role of *Helicobacter pylori* infection in early gastric cancer development. Cancer. 1994;73:2691–4.
29. Van Doorn LJ, Figueiredo C, Mégraud F, Pena S, Midolo P, Queiroz DM, et al. Geographic distribution of *vacA* allelic types of *Helicobacter pylori*. Gastroenterology. 1999;116:823–30.
30. Atherton JC, Peek RM Jr, Tham KT, Cover TL, Blaser MJ. Clinical and pathological importance of heterogeneity in *vacA*, the vacuolating cytotoxin gene of *Helicobacter pylori*. Gastroenterology. 1997;112:92–9.
31. van Doorn LJ, Figueriedo C, Carneiro F, Sanna R, Pena S, Midolo P, et al. Worldwide heterogeneity of the *Helicobacter pylori vacA* gene. Gut. 1997;41 Suppl 1:A34.
32. Atherton JC. The clinical relevance of strain types of *Helicobacter pylori*. Gut. 1997;40:701–3.
33. Palframan SL, Kwok T, Gabriel K. Vacuolating cytotoxin A (*VacA*), a key toxin for *Helicobacter pylori* pathogenesis. Front Cell Infect Microbiol. 2012;12:92.
34. Thi Huyen Trang T, Thanh Binh T, Yamaoka Y. Relationship between vacA types and development of gastroduodenal diseases. Toxins (Basel). 2016;8:182.
35. Alexander SM, Retnakumar RJ, Chouhan D, Devi TNB, Dharmaseelan S, Devadas K, et al. *Helicobacter pylori* in human stomach: the inconsistencies in clinical outcomes and the probable causes. Front Microbiol. 2021;12:713955.
36. Park SK, Kim GH, Jeong EJ, Bae YM, Heo J, Chu HJ, et al. Clinical relevance between the *cagA*, *vacA*, and *iceA* status of *Helicobacter pylori* and benign gastroduodenal diseases. Korean J Gastroenterol. 2002;40:23–31.
37. Cover TL, Blaser MJ. Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. J Biol Chem. 1992;267:10570–5.
38. Nogueira C, Figueiredo C, Carneiro F, Gomes AT, Barreira R, Figueira P, et al. *Helicobacter pylori* genotypes may determine gastric histopathology. Am J Pathol. 2001;158:647–54.
39. Matsunari O, Shiota S, Suzuki R, Watada M, Kinjo N, Murakami K, et al. Association between *Helicobacter pylori* virulence factors and gastroduodenal diseases in Okinawa, Japan. J Clin Microbiol. 2012;50:876–83.
40. Nguyen TL, Uchida T, Tsukamoto Y, Trinh DT, Ta L, Mai BH, et al. *Helicobacter pylori* infection and gastroduodenal diseases in Vietnam: a cross-sectional, hospital-based study. BMC Gastroenterol. 2010;10:114.
41. Sahara S, Sugimoto M, Vilaichone RK, Mahachai V, Miyajima H, Furuta T, et al. Role of *Helicobacter pylori cagA* EPIYA motif and *vacA* genotypes for the development of gastrointestinal diseases in southeast Asian countries: a meta-analysis. BMC Infect Dis. 2012;12:223.
42. Uchida T, Nguyen LT, Takayama A, Okimoto T, Kodama M, Murakami K, et al. Analysis of virulence factors of *Helicobacter pylori* isolated from a Vietnamese population. BMC Microbiol. 2009;9:175.
43. Ogiwara H, Sugimoto M, Ohno T, Vilaichone RK, Mahachai V, Graham DY, et al. Role of deletion located between the intermediate and middle regions of the *Helicobacter pylori vacA* gene in cases of gastroduodenal diseases. J Clin Microbiol. 2009;47:3493–500.
44. Rhead JL, Letley DP, Mohammadi M, Hussein N, Mohagheghi MA, Eshagh Hosseini M, et al. A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. Gastroenterology. 2007;133:926–36.
45. Ogiwara H, Graham DY, Yamaoka Y. *VacA* i-region subtyping. Gastroenterology. 2008;134:1267; author reply 1268.
46. Karbalaei M, Talebi Bezmin Abadi A, Keikha M. Clinical relevance of the cagA and vacA s1m1 status and antibiotic resistance in *Helicobacter pylori*: a systematic review and meta-analysis. BMC Infect Dis. 2022;221:573.
47. van Doorn LJ, Figueiredo C, Sanna R, Plaisier A, Schneeberger P, de Boer W, et al. Clinical relevance of the *cagA*, *vacA*, *and iceA* status of *Helicobacter pylori*. Gastroenterology. 1998;115:58–66.
48. Shiota S, Watada M, Matsunari O, Iwatani S, Suzuki R, Yamaoka Y. *Helicobacter pylori iceA*, clinical outcomes, and correlation with *cagA*: a meta-analysis. PLoS One. 2012;7:e30354.
49. Yamaoka Y, Ojo O, Fujimoto S, Odenbreit S, Haas R, Gutierrez O, et al. *Helicobacter pylori* outer membrane proteins and gastroduodenal disease. Gut. 2006;55:775–81.
50. Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer membrane protein (*oipA*) of *Helicobacter pylori*. Proc Natl Acad Sci U S A. 2000;97:7533–8.
51. Yamaoka Y, Kikuchi S, el-Zimaity HM, Gutierrez O, Osato MS, Graham DY. Importance of *Helicobacter pylori* oipA in clinical presentation, gastric inflammation, and mucosal interleukin 8 production. Gastroenterology. 2002;123:414–24.
52. Liu J, He C, Chen M, Wang Z, Xing C, Yuan Y. Association of presence/absence and on/off patterns of Helicobacter pylori oipA gene with peptic ulcer disease and gastric cancer risks: a meta-analysis. BMC Infect Dis. 2013;13:555.
53. Furuta Y, Kawai M, Yahara K, Takahashi N, Handa N, Tsuru T, et al. Birth and death of genes linked to chromosomal inversion. Proc Natl Acad Sci U S A. 2011;108:1501–6.
54. Kawai M, Furuta Y, Yahara K, Tsuru T, Oshima K, Handa N, et al. Evolution in an oncogenic bacterial species with extreme genome plasticity: Helicobacter pylori east Asian genomes. BMC Microbiol. 2011;11:104.

55. Kim A, Lai J, Merrell DS, Kim JH, Su H, Cha JH. Geographic diversity in *Helicobacter pylori oipA* genotype between Korean and United States isolates. J Microbiol. 2021;59:1125–32.
56. Chen J, Lin L, Li N, She F. Enhancement of *Helicobacter pylori* outer inflammatory protein DNA vaccine efficacy by co-delivery of interleukin-2 and B subunit heat-labile toxin gene encoded plasmids. Microbiol Immunol. 2012;56:85–92.
57. Mahboubi M, Falsafi T, Sadeghizadeh M, Mahjoub F. The role of outer inflammatory protein A (OipA) in vaccination of the C57BL/6 mouse model infected by *Helicobacter pylori*. Turk J Med Sci. 2017;47:326–33.
58. Lu H, Hsu PI, Graham DY, Yamaoka Y. Duodenal ulcer promoting gene of *Helicobacter pylori*. Gastroenterology. 2005;128:833–48.
59. Schmidt HM, Andres S, Kaakoush NO, Engstrand L, Eriksson L, Goh KL, et al. The prevalence of the duodenal ulcer promoting gene (*dupA*) in *Helicobacter pylori* isolates varies by ethnic group and is not universally associated with disease development: a case-control study. Gut Pathog. 2009;1:5.
60. Hussein NR, Argent RH, Marx CK, Patel SR, Robinson K, Atherton JC. *Helicobacter pylori dupA* is polymorphic, and its active form induces proinflammatory cytokine secretion by mononuclear cells. J Infect Dis. 2010;202:261–9.
61. Queiroz DM, Rocha GA, Rocha AM, Moura SB, Saraiva IE, Gomes LI, et al. *DupA* polymorphisms and risk of *Helicobacter pylori*-associated diseases. Int J Med Microbiol. 2011;301:225–8.
62. Talebi Bezmin Abadi A, Perez-Perez G. Role of *dupA* in virulence of *Helicobacter pylori*. World J Gastroenterol. 2016;22:10118–23.
63. Ansari S, Yamaoka Y. *Helicobacter pylori* BabA in adaptation for gastric colonization. World J Gastroenterol. 2017;23:4158–69.
64. Gerhard M, Lehn N, Neumayer N, Borén T, Rad R, Schepp W, et al. Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen-binding adhesin. Proc Natl Acad Sci U S A. 1999;96:12778–83.
65. Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, et al. *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science. 1998;279:373–7.
66. Kim YS, Woo CW, Lee YM, Son BR, Kim JW, Chae HB, et al. Genotyping *CagA*, *VacA* Subtype, *IceA*1, and *BabA* of *Helicobacter pylori* isolates from Korean patients, and their association with gastroduodenal diseases. J Korean Med Sci. 2001;16:579–84.
67. Chen YL, Mo XQ, Huang GR, Huang YQ, Xiao J, Zhao LJ, et al. Gene polymorphisms of pathogenic *Helicobacter pylori* in patients with different types of gastrointestinal diseases. World J Gastroenterol. 2016;22:9718–26.
68. Quintana-Hayashi MP, Rocha R, Padra M, Thorell A, Jin C, Karlsson NG, et al. BabA-mediated adherence of pediatric ulcerogenic *H. pylori* strains to gastric mucins at neutral and acidic pH. Virulence. 2018;9:1699–717.
69. Fujimoto S, Ojo OO, Arnqvist A, Wu JY, Odenbreit S, Haas R, et al. *Helicobacter pylori babA* expression, gastric mucosal injury, and clinical outcome. Clin Gastroenterol Hepatol. 2007;51:49–58.
70. Aspholm-Hurtig M, Dailide G, Lahmann M, Kalia A, Ilver D, Roche N, et al. Functional adaptation of BabA, the *H. pylori* ABO blood group antigen binding adhesin. Science. 2004;305:519–22.
71. Keikha M, Karbalaei M. Correlation between the geographical origin of *Helicobacter pylori homB*-positive strains and their clinical outcomes: a systematic review and meta-analysis. BMC Gastroenterol. 2021;21:181.
72. Alm RA, Bina J, Andrews BM, Doig P, Hancock RE. Comparative genomics of *Helicobacter pylori*: analysis of the outer membrane protein families. Infect Immun. 2000;6:4155–68.
73. Servetas SL, Kim A, Su H, Cha JH, Merrell DS. Comparative analysis of the Hom family of outer membrane proteins in isolates from two geographically distinct regions: the United States and South Korea. Helicobacter. 2018;23:e12461.
74. Oleastro M, Cordeiro R, Ménard A, Gomes JP. Allelic diversity among *Helicobacter pylori* outer membrane protein genes *homB* and *homA* generated by recombination. J Bacteriol. 2010;192:3961–8.
75. Abadi ATB, Rafiei A, Ajami A, Hosseini V, Taghvaei T, Jones KR, et al. *Helicobacter pylori homB*, but not *cagA*, is associated with gastric cancer in Iran. J Clin Microbiol. 2011;49:3191–7.
76. Solnick JV, Hansen LM, Salama NR, Boonjakuakul JK, Syvanen M. Modification of *Helicobacter pylori* outer membrane protein expression during experimental infection of rhesus macaques. Proc Natl Acad Sci U S A. 2004;101:2106–11.
77. Jung SW, Sugimoto M, Graham DY, Yamaoka Y. *homB* status of *Helicobacter pylori* as a novel marker to distinguish gastric cancer from duodenal ulcer. J Clin Microbiol. 2009;47:3241–5.

7

Host Factor: Genetic Polymorphism

Jung Mook Kang and Yonghoon Choi

Abstract

Proinflammatory cytokines and anti-inflammatory cytokines are produced in gastric mucosa from inflammatory cells activated by *Helicobacter pylori* (*H. pylori*) infection. Polymorphisms in these genes are associated with individual differences in cytokine messenger RNA levels, which result in different gastric mucosal inflammations, different acid inhibitions, and different gastroduodenal disease risks in response to *H. pylori* infection. Of the inflammatory cytokines, polymorphisms related to interleukin (*IL*)-*1β* and tumor necrosis factor (*TNF*)-*α*, *IL-10*, and *IL-8* genes have been reported to be associated with the risk of gastric cancer (GC). A functional polymorphism within toll-like receptors (*TLRs*) and nucleotide-binding oligomerization domain (*NOD*)-like receptors (*NLRs*) has also been demonstrated to increase the risk for gastric atrophy and GC. However, the prevalence of cytokine gene genotypes differs between Western and Asian populations, and the role of genetic polymorphism on GC related to *H. pylori* might be different between race and geographic region.

Keywords

Genetic polymorphism · Cytokine
Helicobacter pylori

7.1 Introduction

Helicobacter pylori (*H. pylori*) infects over half of the world's population, but there is variation in incidence among different geographic regions. Eighty-five percent of *H. pylori*-infected individuals remain lifelong asymptomatic, while only 1% of these individuals develop gastric cancer (GC) and 10% develop peptic ulcer [1]. Indeed, the clinical consequences of infection by *H. pylori* are determined by multiple factors, including genetic predisposition of the host, especially regarding certain cytokine, and receptor gene polymorphisms, gene regulation, environmental factors such as high dietary salt intake, and heterogeneity of *H. pylori* strains [2] (Fig. 7.1). Among them, host genetic factors that affect cytokine polymorphisms may determine why some individuals infected with *H. pylori* develop GC while others do not. The inflammatory-related genes that have been most frequently studied in relation to GC, sometimes with conflicting results, are the gene-related interleukin (*IL*)-1β, tumor necrosis factor (*TNF*)-α, *IL-10,*

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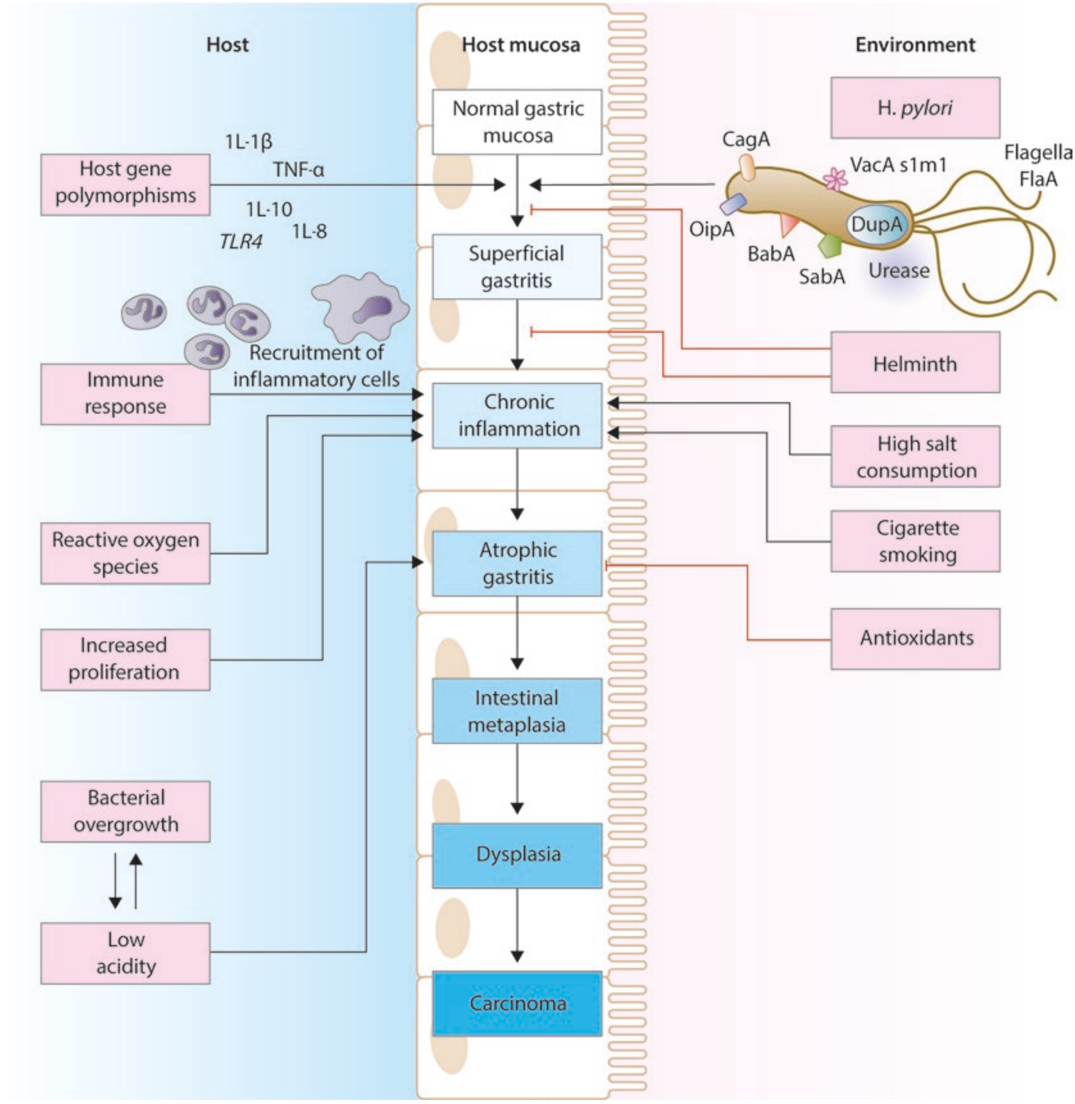
Host
Host mucosa
Environment
Normal gastric mucosa
H. *pylori*
CagA
VacA s1m1
Flagella FlaA
1L-1β
TNF-α
Host gene polymorphisms
1L-10
TLR4
1L-8
OipA
BabA
DupA
SabA
Urease
Superficial gastritis
Recruitment of inflammatory cells
Immune response
Helminth
Chronic inflammation
High salt consumption
Reactive oxygen species
Cigarette smoking
Atrophic gastritis
Antioxidants
Increased proliferation
Intestinal metaplasia
Bacterial overgrowth
Dysplasia
Low acidity
Carcinoma

Fig. 7.1 Multifactorial pathway leading to gastric cancer. Many host, bacterial, and environmental factors act in combination to contribute to the precancerous cascade leading to the development of gastric cancer (Adapted from Wroblewski et al. [2])

IL-8, toll-like receptors (*TLRs*), and nucleotide-binding oligomerization domain (*NOD*)-like receptors (*NLRs*). Single nucleotide polymorphisms (SNPs) within these and other functional cytokine regions that markedly influence expression and secretion profiles may modify the intensity of the inflammatory response to infectious agents, thereby contributing to variations in GC risk [3–28] (Table 7.1).

Table 7.1 Host genetic polymorphism that has been associated with the development of gastric cancer

Protein/effectors	Polymorphism	Effect	References
Interleukin 1β	*IL-1B*-31 C *IL-1B*-511 T *IL-1RN* *2	High-level expression of IL-1β, reduction of acid output, corpus colonization by *H. pylori* *IL-1B*-31 C, *IL-1B*-511 T, and *IL-1RN* *2 alleles are associated to an increased risk of gastric cancer	[3–9]
Tumor necrosis factor-α	*TNF*-α-308 *TNF*-α-238 *TNF*-α-857	High-level expression of TNF-α Increased risk of gastric cancer in Caucasians Increased risk of gastric tumorigenesis in Asians, but not in Caucasians Increased risk of gastric tumorigenesis in Asians, but not in Caucasians	[10–13]
Interleukin-10	*IL-10*-1082 G/A *IL-10*-819 C/T *IL-10*-592 C/A *IL-10* ATA *IL-10* GCC	Low secretion of IL-10 is associated with high inflammation and high risk to gastric cancer Haplotype ATA is low IL-10 secreting and haplotype GCC is high IL-10 secreting	[14, 15]
Interleukin-8	*IL-8*-251	High IL-8 levels are found in gastric cancer *IL-8*-251 A allele is associated to a higher production of IL-8	[15–20]
Toll-like receptors	*TLR4* Asp299Gly *TLR4* Thr399Ile	TLR-4 is associated with hyporesponsiveness to LPS and therefore to *H. pylori*	[21, 22]
NOD-like receptors	*NOD1 rs2709800* *NOD1 rs2907749* *NOD1 rs7789045* *NOD2 rs718226* *NOD2 rs2111235* *NOD2 rs7205423* *NOD2 rs2066842* P268S *NOD2 rs2066844* R702W *NOD2 rs7202124*	GT genotype increases risk of intestinal metaplasia G-allele is associated with a decreased risk of gastric cancer TT genotype is associated with increased risk of gastric cancer G allele is associated with greater risk of dysplasia C allele increases risk of disease progression in *H. pylori* infection, associated with a decreased risk in European populations G allele increases risk of disease progression in *H. pylori* infection T-allele is associated with increased risk of gastric cancer T-allele is associated with increased risk of gastric cancer G-allele is associated with decreased risk of gastric cancer	[23] [24] [24] [23] [25] [24] [26, 27] [28] [25]

NOD nucleotide-binding oligomerization domain

7.2 Interleukin-1β

IL-1β is a Th1 cytokine that inhibits acid secretion and is increased within gastric mucosa of *H. pylori*-infected persons [29]. IL-1β is 100 times a more potent inhibitor than a proton pump inhibitor (PPI) and 6000 times more potent than histamine-2 receptor antagonists on a molar basis [30, 31]. In animal studies, decreased acid secretion is reported to be accompanied by an elevation of IL-1β mRNA levels in the *H. pylori*-infected gastric mucosa [32].

IL-1β is encoded by *IL-1B* on chromosome 2q13-14, which has polymorphisms at positions *IL-1B*-511 and *IL-1B*-31 base pairs (bp) from the transcriptional start site [33]. The gene for *IL-1 receptor antagonist* (*IL-1RN*) has a variable number tandem repeats (VNTR) of 86 bp in length in intron 2 [34, 35] (Fig. 7.2). The carriers of the *IL-1B*-511 T, *IL-1B*-31 C, and *IL-RN* *2 (2 repeats of 86 bp) alleles have significantly higher IL-1β levels than carriers of the other genotype [3] (Fig. 7.3). Consistent with this difference, carriers of the *IL-1B*-511 T, *IL-1B*-31 C alleles,

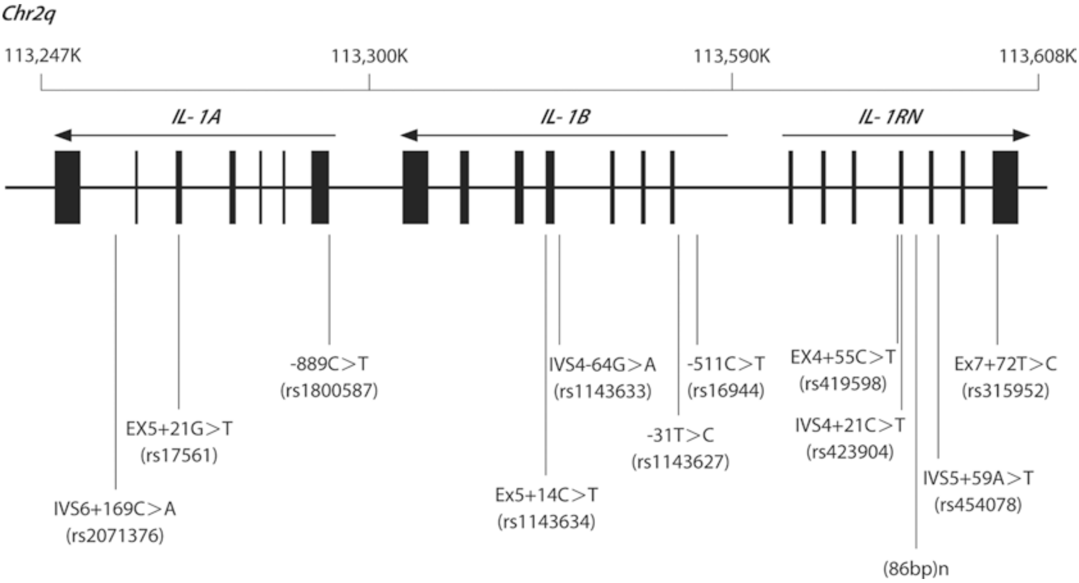
Chr2q
113,247K
113,300K
113,590K
113,608K
IL-1A
IL-1B
IL-1RN
IVS6+169C>A (rs2071376)
EX5+21G>T (rs17561)
-889C>T (rs1800587)
Ex5+14C>T (rs1143634)
IVS4-64G>A (rs1143633)
-31T>C (rs1143627)
-511C>T (rs16944)
EX4+55C>T (rs419598)
IVS4+21C>T (rs423904)
(86bp)n
IVS5+59A>T (rs454078)
Ex7+72T>C (rs315952)

Fig. 7.2 *IL-1A*, *IL-1B*, and *IL-1RN*, showing polymorphic nucleotide sequences (Adapted from Kim et al. [35])

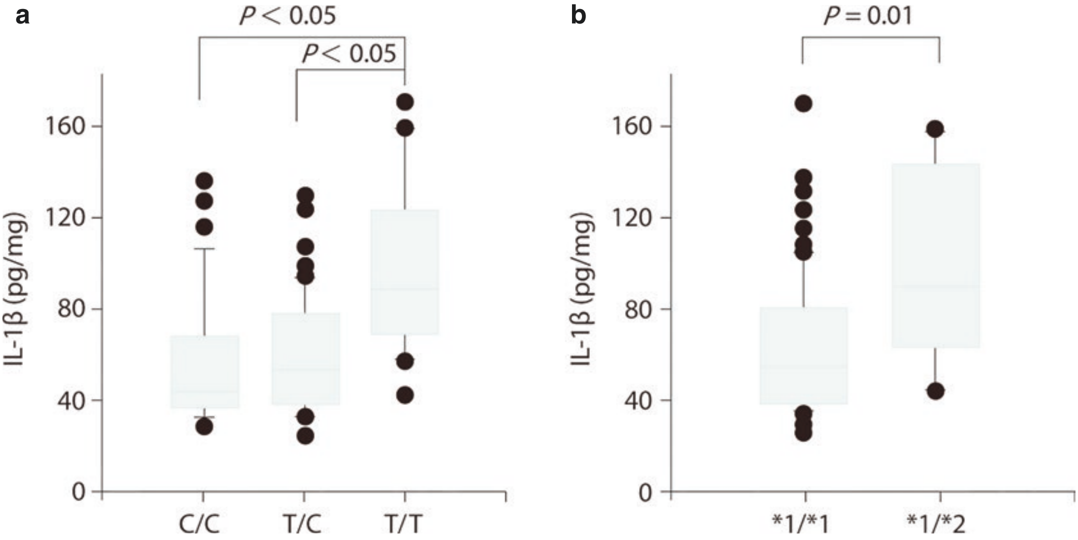

Fig. 7.3 Mucosal IL-1β levels in relation to the genotypes at (**a**) *IL-1B*-511 and (**b**) *IL-1RN* (Modified from Hwang et al. [3])

and *IL-1RN* *2 genotype show enhanced suppression of gastric acid secretion, which results in more rapid development of gastric atrophy and a consequently greater risk of developing GC than in those with the *IL-1B*-511 C, *IL-1B*-31 T, and *IL-1RN* *1 alleles [3–8]. The meta-analysis by Xue et al. [36] showed that *IL-1B*-511 T and *IL-1RN* *2 alleles were significantly associated with an increased risk of developing GC among Caucasians, but not in Asians. A recent meta-analysis by Ma et al. [9] also suggested that *IL-1B*-511 T allele might act as a risk factor to *H. pylori*-related GC or peptic ulcer diseases. Another meta-analysis by Persson et al. [37] revealed a consistent negative association of *IL-1B*-31 C allele with GC in Asians. These results suggest that this divergence may reflect the different genetic background related to ethnicity.

7.3 Tumor Necrosis Factor-α

TNF-α is a proinflammatory cytokine induced by *H. pylori* and inhibits gastric acid secretion [38, 39]. TNF-α mRNA and protein expressions were significantly increased in GC patients [40]. *TNF*-α encoding TNF-α is located on chromosome 6 (6p21). Among the many polymorphisms in *TNF-α* promoter, the *TNF-α*-308 G/A polymorphism was wildly studied, and there is functional study suggesting that the *TNF-α*-308 A allele is associated with increased TNF-α production [10, 11]. To date, several molecular epidemiological studies have been conducted to investigate the association between the *TNF-α*-308 G/A genotype and GC risk. There are two published meta-analyses on the associations between the *TNF-α*-308 A allele and GC risk that have identified this polymorphism as a risk factor for GC in Caucasian populations but not in Asian populations [41, 42]. Actually, these polymorphisms are not found in Asian populations because most of their patients have lower producer alleles, *TNF-α*-308 G/G genotypes [8, 43, 44]. A recent Moroccan study reported that the *TNF-α*-308 A allele has a promotive effect for GC progression, whereas the *TNF-α*-238 A allele has a protective function against GC progression, that higher levels of TNF-α seemed to be associated with the aggressiveness of gastric lesions [12]. In contrast, another meta-analysis suggested that *TNF-α*-308 G/A was significantly associated with GC only in Caucasians, while *TNF-α*-857 and *TNF-α*-238 were related to an increased risk of gastric tumorigenesis in Asians, but not in Caucasians [13]. Put together, the results from studies so far remain inconclusive.

7.4 Interleukin-10

IL-10 is an anti-inflammatory cytokine that downregulates cell-mediated immune responses and cytotoxic inflammatory responses [45, 46]. *H. pylori* can lead to IL-10 upregulation as a way to suppress an efficient immune response, which then favors infection and parasite survival [47]. IL-10 levels are elevated in gastric mucosa infected with *H. pylori* and are higher in patients that have severe chronic inflammation [48]. Furthermore, IL-10 mRNA expression and serum levels are elevated in GC, particularly in the advanced stage [49].

The gene encoding IL-10 is located on chromosome 1 (1q31–32). There are three functional promoter SNPs in the *IL-10* locus: *IL-10*-1082 G/A, *IL-10*-819 C/T, and *IL-10*-592 C/A. It has been reported that these variants (GCC carriers) of *IL-10*-1082/*IL-10*-819/*IL-10*-592 are associated with increased IL-10 production [50]. The low IL-10 production is associated with increased gastric inflammation intensity and with an enhanced risk of GC in patients infected with *H. pylori* [2]. However, high IL-10 producer genotype was significantly increased in GC patients from Taiwan and China [14, 15]. In addition, recent meta-analyses suggested that Asian carriers of the promoter polymorphisms of *IL-10*-592 C and *IL-10*-1082 G alleles may be associated with an increased risk of GC [51, 52]. In contrast to Asian populations, mostly studies conducted in Western populations have reported a different association. That is there was an association identified between the low IL-10 producer (*IL-10*-592 A allele) and the risk of GC in populations from the United States and Italy [53, 54]. Several meta-analyses have been performed to evaluate the association between *IL-10* polymorphisms and cancer risk [55–58]. When stratifying the data by race, the low IL-10 producer was found to be a protective factor against the development of this neoplasm in Asians but not among Western populations suggesting a possible role of ethnic differences in genetic backgrounds and the environment they lived in.

7.5 Interleukin-8

IL-8, a member of the CXC chemokine family, which was originally identified as a potent chemoattractant for neutrophils and lymphocytes, induces not only cell proliferation and migration but also angiogenesis. The high expression of IL-8 has been demonstrated in gastric mucosa infected with *H. pylori* [59]. IL-8 causes chemotaxis and the activation of inflammatory cells in gastric mucosa infected with *H. pylori* [60]. In addition, IL-8 protein levels are tenfold higher in GC than in normal gastric tissue [61] and directly correlate with the vascularity of the tumors [62].

The *IL-8* gene, which is located on chromosome 4q12–21, exhibits SNP T-A base transition at 251 bp from the transcription start site [63]. *IL-8*-251 A alleles are associated with higher IL-8 production, and consequently with increased mucosal injury, by activating and recruiting neutrophils in response to infection by *H. pylori* [54, 64]. *H. pylori*-positive patients and carriers of the *IL-8*-251 A/A genotype at position had an increased risk of peptic ulcer disease [16, 17]. Most studies that have reported positive associations for GC risk have been conducted in Asian populations [15–20], whereas those that have reported negative findings were conducted in Western populations [65, 66]. Moreover, a recent meta-analysis suggests the potential influence of ethnicity in the association of *IL-8*-251 A/T genotype with GC since it is generally stronger in Asian than in Caucasian population [67].

7.6 Toll-Like Receptor 4

TLRs are critical for pathogen detection and downstream signaling to stimulate effective immunity [68, 69], and *H. pylori* factors such as lipopolysaccharide (LPS), NapA, HSP-60, RNA, and DNA were reported to be recognized by specific TLRs such as TLR1, TLR2, TLR4, TLR6, TLR9, and TLR10, while *H. pylori* flagellin was found to evade recognition by TLR5. TLR4, which constitutes one of the most active members of TLRs, promotes the transcription of genes involved in immune activation [70]. TLR4 was initially identified as the potential signaling receptor for *H. pylori* on gastric epithelial cells [71], and from a theoretical point of view, the activation of TLR4 may irritate the immune response that protects the organism against tumors, produce a proinflammatory environment, and, thus, may promote carcinogenesis.

Overexpression of various TLRs has been found in precancerous and cancerous gastric tissues [72], and *TLR4* rs4986790 (Asp299Gly), *TLR4* rs4986791 (Thr399Ile), *TLR4* rs10116253, *TLR4* rs10983755, *TLR4* rs11536889 (c.3725G/C), and *TLR4* rs1927911 are most notably identified to be associated with GC [73–75]. Among these polymorphisms, Asp299Gly and Thr399Ile are most well known to be correlated with a blunted response to LPS in vivo and in vitro [21, 22] and to be linked with susceptibility to *H. pylori* infection, atrophic gastritis, and GC in Caucasian populations [76, 77]. Asp299Gly polymorphism was found to be a risk factor for non-cardia GC and its precursors including achlorhydria in Poland, Scotland, and the United States population [76]. Recent meta-analysis including 12 case–control studies demonstrated a significant correlation between the G allele of the Asp299Gly polymorphism and increased risk of GC but not in Thr399Ile polymorphism [78]. However, there are significant differences in the intercontinental incidence of these two SNPs. Actually, in Asian populations at relatively high risk of *H. pylori* infection and its associated GC, such as Chinese, Japanese, and Korean populations, the two *TLR4* polymorphisms are almost never found [79–81]. In addition, CD14 (cluster of differentiation 14) is a factor that co-operates with TLR4 in the recognition of LPS, and two SNPs in the promoter region of *CD14* (-260C/T, rs2569190 and -651C/T, rs5744455) have been found to be associated with *H. pylori*-related risk of GC development [73, 82].

7.7 Nucleotide-Binding Oligomerization Domain-Like Receptors (NLRs)

NLRs are conserved intracellular sensors of bacterially released peptidoglycan [83] and play fundamental and pleiotropic roles in host defense against bacterial infections and in the control of inflammation. To date, the best characterized of these are NOD1 and NOD2 (also known as CARD4 and CARD15, respectively) [84]. Overall, a reduced NOD1/2-mediated immune response to *H. pylori* contributes to bacterial persistence, and a subsequent overactivation of other inflammatory responses may result in inflammation-related carcinogenesis [84].

Several SNPs in *NOD1* and *NOD2* have been reported to be associated with GC in various studies and populations, but interpretation is complicated so far. For rs2709800, one SNP in *NOD1*, the heterozygous G/T genotype was associated with increased intestinal metaplasia [23], which is difficult to understand as homozygotes would be expected to have a higher impact on disease. Another study showed that homozygotes of the *NOD1* rs2907749 G allele had decreased risk of GC, while the *NOD1* rs7789045 T/T genotype carriers were associated with increased risk [24]. Genetic variation at the *NOD2* locus was more studied than NOD1 since NOD2 variations are known to be associated with other diseases such as inflammatory bowel disease (IBD) and leprosy [85]. The *NOD2* rs718226 G allele showed an increased risk of gastric dysplasia and cancer in a Chinese study, whereas the *NOD2* rs2111235 C allele and rs7205423 G allele showed a decreased risk of disease progression in *H. pylori*-infected patients [23]. In another study, an association was found between the *NOD2* rs7205423 G/C genotype and GC susceptibility in *H. pylori*-positive Chinese patients [24]. The *NOD2* rs2111235 as well as rs7202124 turned out to be protective for GC in a European cohort study [25], whereas an association between NOD2 and GC risk was described for rs2066842 (c.802C>T) in German [26] and Polish [27] studies and rs2066844 (c.2104C>T) in an Italian study [28]. Since the results are somewhat inconsistent and the potential biological consequences of these SNPs are unknown, yet, understanding the influence of host genetic factors on NOD signaling remains challenging. A schematic overview of interaction of *H. pylori* with various host factors via TLRs and NLRs in the pathogenesis of GC is presented in Fig. 7.4 [84].

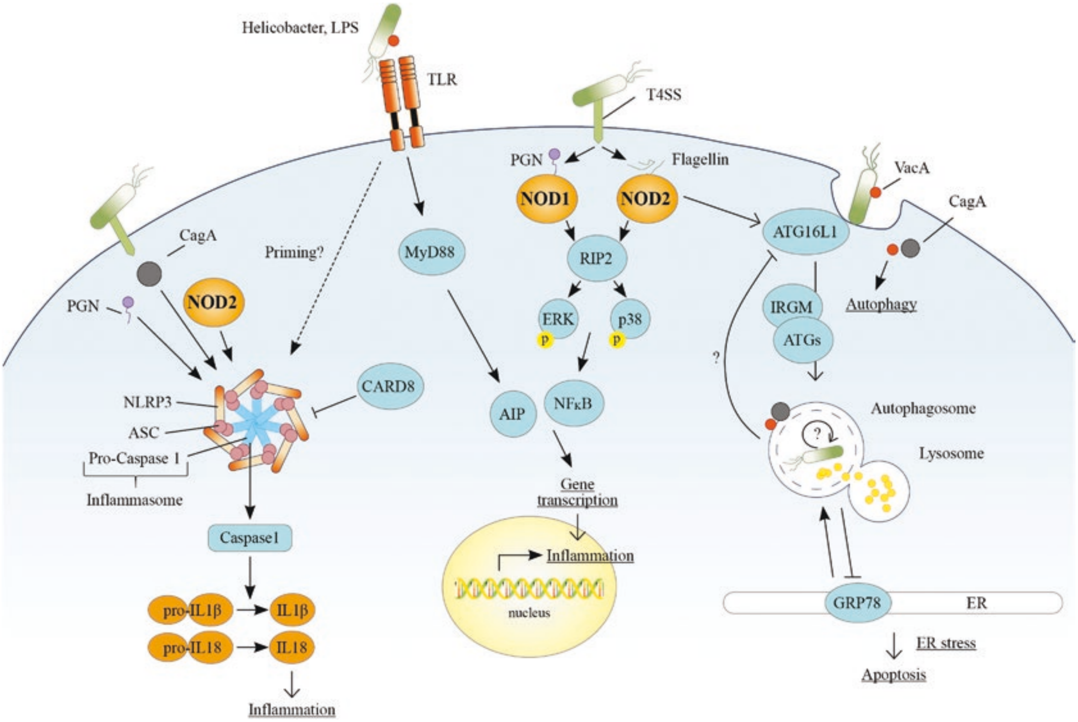
Helicobacter, LPS
TLR
T4SS
PGN
Flagellin
VacA
CagA
NOD1
NOD2
ATG16L1
CagA
Priming?
MyD88
RIP2
PGN
NOD2
IRGM
Autophagy
ERK
p38
P
P
ATGs
?
NLRP3
ASC
Pro-Caspase 1
Inflammasome
CARD8
AIP
NFκB
Autophagosome
?
Lysosome
Gene transcription
Caspase1
Inflammation
nucleus
pro-IL1β
IL1β
pro-IL18
IL18
GRP78
ER
ER stress
Apoptosis
Inflammation

Fig. 7.4 Schematic representation of the interaction of *H. pylori* with various host factors implicated in the pathogenesis of gastric cancer (Adapted from Mommersteeg et al. [84]). *TLR* Toll-like receptor, *LPS* lipopolysaccharide, *PGN* peptidoglycan, *VacA* vacuolating cytotoxin A, *CagA* cytotoxin-associated gene A, *T4SS* type IV secretion system, *RIP2* receptor protein-interacting 2, *CARD8* caspase recruitment domain-containing protein 8, *NFκB* nuclear factor kappa B, *AIP* activator protein 1, *ERK* extracellular signal regulated kinase, *ROS* reactive oxygen species, *NADPH* nicotinamide adenine dinucleotide phosphate, *ER* endoplasmic reticulum

7.8 Conclusions

Currently, many epidemiologic studies have demonstrated that GC has a multifactorial etiology and is comodulated by different factors including *H. pylori* infection, lifestyle, socioeconomic status, and environmental factors. In addition, genetic factors are increasingly recognized as major contributors to GC risk. Recent studies have reported the association between *IL-1B*, *TNF-α*, *IL-10*, and *IL-8* polymorphisms and the development of GC. Furthermore, a functional polymorphism within *TLRs* and *NLRs* has also been demonstrated to increase the risk for gastric atrophy and GC. However, the prevalence of cytokine gene genotypes differs between Western and Asian populations, and the role of genetic polymorphism on GC related to *H. pylori* might be different between race and geographic region. Thus, more large population studies for the analysis of genetic polymorphisms in relation to *H. pylori*-related disease are needed involving bacterial factor and environmental variables (lifestyle, dietary habits) in order to contribute to the understanding of gastric carcinogenesis and for the development of screening programs to prevent GC.

References

1. Peek RM Jr, Crabtree JE. Helicobacter infection and gastric neoplasia. J Pathol. 2006;208:233–48.
2. Wroblewski LE, Peek RM Jr, Wilson KT. *Helicobacter pylori* and genetic polymorphism: factors that modulate disease risk. Clin Microbiol Rev. 2010;23:713–39.
3. Hwang IR, Kodama T, Kikuchi S, Sakai K, Peterson LE, Graham DY, et al. Effect of interleukin 1 polymorphisms on gastric mucosal interleukin 1beta production in *Helicobacter pylori* infection. Gastroenterology. 2002;123:1793–803.
4. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature. 2000;404:398–402.
5. Take S, Mizuno M, Ishiki K, Nagahara Y, Yoshida T, Inaba T, et al. Interleukin-1beta genetic polymorphism influences the effect of cytochrome P 2C19 genotype on the cure rate of 1-week triple therapy for *Helicobacter pylori* infection. Am J Gastroenterol. 2003;98:2403–8.
6. Furuta T, Shirai N, Takashima M, Xiao F, Sugimura H. Effect of genotypic differences in interleukin-1 beta on gastric acid secretion in Japanese patients infected with *Helicobacter pylori*. Am J Med. 2002;112:141–3.
7. Machado JC, Pharoah P, Sousa S, Carvalho R, Oliveira C, Figueiredo C, et al. Interleukin 1B and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. Gastroenterology. 2001;121:823–9.
8. Sugimoto M, Furuta T, Shirai N, Nakamura A, Xiao F, Kajimura M, et al. Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcer and gastric cancer. J Gastroenterol Hepatol. 2007;22:51–9.
9. Ma J, Wu D, Hu X, Li J, Cao M, Dong W. Associations between cytokine gene polymorphisms and susceptibility to *Helicobacter pylori* infection and *Helicobacter pylori* related gastric cancer, peptic ulcer disease: a meta-analysis. PLoS One. 2017;12:e0176463.
10. Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. Mol Immunol. 1997;34:391–9.
11. Wilson AG, di Giovine FS, Blakemore AI, Duff GW. Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. Hum Mol Genet. 1992;1:353.
12. Bounder G, Jouimyi MR, Boura H, Touati E, Michel V, Badre W, et al. Associations of the -238(G/A) and -308(G/A) TNF-α promoter polymorphisms and TNF-α serum levels with the susceptibility to gastric precancerous lesions and gastric cancer related to *Helicobacter pylori* infection in a Moroccan population. Asian Pac J Cancer Prev. 2020;21:1623–9.
13. Zhao H, Liu L, Liu B, Wang Y, Li F, Yu H. An updated association between TNF-α -238G/A polymorphism and gastric cancer susceptibility in East Asians. Biosci Rep. 2018;38:BSR20181231.
14. Wu MS, Wu CY, Chen CJ, Lin MT, Shun CT, Lin JT. Interleukin-10 genotypes associate with the risk of gastric carcinoma in Taiwanese Chinese. Int J Cancer. 2003;104:617–23.
15. Lu W, Pan K, Zhang L, Lin D, Miao X, You W. Genetic polymorphisms of interleukin (IL)-1B, IL-1RN, IL-8, IL-10 and tumor necrosis factor {alpha} and risk of gastric cancer in a Chinese population. Carcinogenesis. 2005;26:631–6.
16. Ohyauchi M, Imatani A, Yonechi M, Asano N, Miura A, Iijima K, et al. The polymorphism interleukin 8–251 A/T influences the susceptibility of *Helicobacter pylori* related gastric diseases in the Japanese population. Gut. 2005;54:330–5.
17. Kang JM, Kim N, Lee DH, Park JH, Lee MK, Kim JS, et al. The effects of genetic polymorphisms of IL-6, IL-8, and IL-10 on *Helicobacter pylori*-induced gastroduodenal diseases in Korea. J Clin Gastroenterol. 2009;43:420–8.

18. Taguchi A, Ohmiya N, Shirai K, Mabuchi N, Itoh A, Hirooka Y, et al. Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan. Cancer Epidemiol Biomarkers Prev. 2005;14(11 Pt 1):2487–93.
19. Savage SA, Abnet CC, Mark SD, Qiao YL, Dong ZW, Dawsey SM, et al. Variants of the IL8 and IL8RB genes and risk for gastric cardia adenocarcinoma and esophageal squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2004;13:2251–7.
20. Ye BD, Kim SG, Park JH, Kim JS, Jung HC, Song IS. The interleukin-8-251 A allele is associated with increased risk of noncardia gastric adenocarcinoma in *Helicobacter pylori*-infected Koreans. J Clin Gastroenterol. 2009;43:233–9.
21. Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet. 2000;25:187–91.
22. Norata GD, Garlaschelli K, Ongari M, Raselli S, Grigore L, Benvenuto F, et al. Effect of the toll-like receptor 4 (TLR-4) variants on intima-media thickness and monocyte-derived macrophage response to LPS. J Intern Med. 2005;258:21–7.
23. Li ZX, Wang YM, Tang FB, Zhang L, Zhang Y, Ma JL, et al. NOD1 and NOD2 genetic variants in association with risk of gastric cancer and its precursors in a Chinese population. PLoS One. 2015;10:e0124949.
24. Wang P, Zhang L, Jiang JM, Ma D, Tao HX, Yuan SL, et al. Association of NOD1 and NOD2 genes polymorphisms with *Helicobacter pylori* related gastric cancer in a Chinese population. World J Gastroenterol. 2012;18:2112–20.
25. Companioni O, Bonet C, Muñoz X, Weiderpass E, Panico S, Tumino R, et al. Polymorphisms of *Helicobacter pylori* signaling pathway genes and gastric cancer risk in the European Prospective Investigation into Cancer-Eurgast cohort. Int J Cancer. 2014;134:92–101.
26. Wex T, Ebert MPA, Kropf S, Dierkes J, Schüttler K, Röcken C, et al. Gene polymorphisms of the NOD-2/CARD-15 gene and the risk of gastric cancer in Germany. Anticancer Res. 2008;28:757–62.
27. Hnatyszyn A, Szalata M, Stanczyk J, Cichy W, Slomski R. Association of c.802C>T polymorphism of NOD2/CARD15 gene with the chronic gastritis and predisposition to cancer in *H. pylori* infected patients. Exp Mol Pathol. 2010;88:388–93.
28. Angeletti S, Galluzzo S, Santini D, Ruzzo A, Vincenzi B, Ferraro E, et al. NOD2/CARD15 polymorphisms impair innate immunity and increase susceptibility to gastric cancer in an Italian population. Hum Immunol. 2009;70:729–32.
29. Noach LA, Bosma NB, Jansen J, Hoek FJ, van Deventer SJ, Tytgat GN. Mucosal tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-8 production in patients with *Helicobacter pylori* infection. Scand J Gastroenterol. 1994;29:425–9.
30. Wallace JL, Cucala M, Mugridge K, Parente L. Secretagogue-specific effects of interleukin-1 on gastric acid secretion. Am J Physiol. 1991;261(4 Pt 1):G559–64.
31. Wolfe MM, Nompleggi DJ. Cytokine inhibition of gastric acid secretion—a little goes a long way. Gastroenterology. 1992;102:2177–8.
32. Takashima M, Furuta T, Hanai H, Sugimura H, Kaneko E. Effects of *Helicobacter pylori* infection on gastric acid secretion and serum gastrin levels in Mongolian gerbils. Gut. 2001;48:765–73.
33. Stokkers PC, van Aken BE, Basoski N, Reitsma PH, Tytgat GN, van Deventer SJ. Five genetic markers in the interleukin 1 family in relation to inflammatory bowel disease. Gut. 1998;43:33–9.
34. Tarlow JK, Blakemore AI, Lennard A, Solari R, Hughes HN, Steinkasserer A, et al. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet. 1993;91:403–4.
35. Kim SH, Mok JW, Kim HS, Joo CK. Association of -31T>C and -511 C>T polymorphisms in the interleukin 1 beta (IL1B) promoter in Korean keratoconus patients. Mol Vis. 2008;14:2109–16.
36. Xue H, Lin B, Ni P, Xu H, Huang G. Interleukin-1B and interleukin-1 RN polymorphisms and gastric carcinoma risk: a meta-analysis. J Gastroenterol Hepatol. 2010;25:1604–17.
37. Persson C, Canedo P, Machado JC, El-Omar EM, Forman D. Polymorphisms in inflammatory response genes and their association with genetic polymorphism: a HuGE systematic review and meta-analyses. Am J Epidemiol. 2011;173:259–70.
38. Jung HC, Kim JM, Song IS, Kim CY. *Helicobacter pylori* induces an array of pro-inflammatory cytokines in human gastric epithelial cells: quantification of mRNA for interleukin-8, -1 alpha/beta, granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein-1 and tumour necrosis factor-alpha. J Gastroenterol Hepatol. 1997;12:473–80.
39. Beales IL, Calam J. Interleukin 1 beta and tumour necrosis factor alpha inhibit acid secretion in cultured rabbit parietal cells by multiple pathways. Gut. 1998;42:227–34.
40. Senthilkumar C, Niranjali S, Jayanthi V, Ramesh T, Devaraj H. Molecular and histological evaluation of tumor necrosis factor-alpha expression in *Helicobacter pylori*-mediated gastric carcinogenesis. J Cancer Res Clin Oncol. 2011;137:577–83.
41. Zhang J, Dou C, Song Y, Ji C, Gu S, Xie Y, et al. Polymorphisms of tumor necrosis factor-alpha are associated with increased susceptibility to genetic polymorphism: a meta-analysis. J Hum Genet. 2008;53:479–89.
42. Gorouhi F, Islami F, Bahrami H, Kamangar F. Tumour-necrosis factor-A polymorphisms and gastric cancer risk: a meta-analysis. Br J Cancer. 2008;98:1443–51.
43. Ohyama I, Ohmiya N, Niwa Y, Shirai K, Taguchi A, Itoh A, et al. The association between tumour necrosis factor-alpha gene polymorphism and the susceptibility to rugal hyperplastic gastritis and gastric carcinoma. Eur J Gastroenterol Hepatol. 2004;16:693–700.

44. Kim N, Cho SI, Yim JY, Kim JM, Lee DH, Park JH, et al. The effects of genetic polymorphisms of IL-1 and TNF-A on *Helicobacter pylori*-induced gastroduodenal diseases in Korea. Helicobacter. 2006;11:105–12.
45. Mosser DM, Zhang X. Interleukin-10: new perspectives on an old cytokine. Immunol Rev. 2008;226:205–18.
46. Kim J, Cho YA, Choi IJ, Lee YS, Kim SY, Shin A, et al. Effects of interleukin-10 polymorphisms, *Helicobacter pylori* infection, and smoking on the risk of noncardia gastric cancer. PLoS One. 2012;7:e29643.
47. Assis S, Marques CR, Silva TM, Costa RS, Alcantara-Neves NM, Barreto ML, et al. IL10 single nucleotide polymorphisms are related to upregulation of constitutive IL-10 production and susceptibility to *Helicobacter pylori* infection. Helicobacter. 2014;19:168–73.
48. Bodger K, Wyatt JI, Heatley RV. Gastric mucosal secretion of interleukin-10: relations to histopathology, *Helicobacter pylori* status, and tumour necrosis factor-alpha secretion. Gut. 1997;40:739–44.
49. De Vita F, Orditura M, Galizia G, Romano C, Infusino S, Auriemma A, et al. Serum interleukin-10 levels in patients with advanced gastrointestinal malignancies. Cancer. 1999;86:1936–43.
50. Suárez A, Castro P, Alonso R, Mozo L, Gutiérrez C. Interindividual variations in constitutive interleukin-10 messenger RNA and protein levels and their association with genetic polymorphisms. Transplantation. 2003;75:711–7.
51. Zhuang W, Wu XT, Zhou Y, Liu L, Liu GJ, Wu TX, et al. Interleukin10-592 promoter polymorphism associated with gastric cancer among Asians: a meta-analysis of epidemiologic studies. Dig Dis Sci. 2010;55:1525–32.
52. Zhou Y, Li N, Zhuang W, Liu GJ, Wu TX, Yao X, et al. Interleukin-10 -1082 promoter polymorphism associated with gastric cancer among Asians. Eur J Cancer. 2008;44:2648–54.
53. El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, et al. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. Gastroenterology. 2003;124:1193–201.
54. Zambon CF, Basso D, Navaglia F, Belluco C, Falda A, Fogar P, et al. Pro- and anti-inflammatory cytokines gene polymorphisms and *Helicobacter pylori* infection: interactions influence outcome. Cytokine. 2005;29:141–52.
55. Yu Z, Liu Q, Huang C, Wu M, Li G. The interleukin 10–819C/T polymorphism and cancer risk: a HuGE review and meta-analysis of 73 studies including 15,942 cases and 22,336 controls. OMICS. 2013;17:200–14.
56. Xue H, Wang YC, Lin B, An J, Chen L, Chen J, et al. A meta-analysis of interleukin-10 -592 promoter polymorphism associated with gastric cancer risk. PLoS One. 2012;7:e39868.
57. Li C, Tong W, Liu B, Zhang A, Li F. The -1082A>G polymorphism in promoter region of interleukin-10 and risk of digestive cancer: a meta-analysis. Sci Rep. 2014;4:5335.
58. Zhu Y, Wang J, He Q, Zhang JQ. The association between interleukin-10-592 polymorphism and gastric cancer risk: a meta-analysis. Med Oncol. 2011;28:133–6.
59. Kusugami K, Ando T, Ohsuga M, Imada A, Shinoda M, Konagaya T, et al. Mucosal chemokine activity in *Helicobacter pylori* infection. J Clin Gastroenterol. 1997;25 (Suppl 1):S203–10.
60. Naito M, Eguchi H, Goto Y, Kondo T, Nishio K, Ishida Y, et al. Associations of plasma IL-8 levels with *Helicobacter pylori* seropositivity, gastric atrophy, and IL-8 T-251A genotypes. Epidemiol Infect. 2010;138:512–8.
61. Yamaoka Y, Kodama T, Kita M, Imanishi J, Kashima K, Graham DY. Relation between cytokines and *Helicobacter pylori* in gastric cancer. Helicobacter. 2001;6:116–24.
62. Kitadai Y, Takahashi Y, Haruma K, Naka K, Sumii K, Yokozaki H, et al. Transfection of interleukin-8 increases angiogenesis and tumorigenesis of human gastric carcinoma cells in nude mice. Br J Cancer. 1999;81:647–53.
63. Hull J, Thomson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. Thorax. 2000;55:1023–7.
64. Li ZW, Wu Y, Sun Y, Liu LY, Tian MM, Feng GS, et al. Inflammatory cytokine gene polymorphisms increase the risk of atrophic gastritis and intestinal metaplasia. World J Gastroenterol. 2010;16:1788–94.
65. Kamangar F, Abnet CC, Hutchinson AA, Newschaffer CJ, Helzlsouer K, Shugart YY, et al. Polymorphisms in inflammation-related genes and risk of gastric cancer (Finland). Cancer Causes Control. 2006;17:117–25.
66. Savage SA, Hou L, Lissowska J, Chow WH, Zatonski W, Chanock SJ, et al. Interleukin-8 polymorphisms are not associated with gastric cancer risk in a Polish population. Cancer Epidemiol Biomarkers Prev. 2006;15:589–91.
67. Cheng D, Hao Y, Zhou W, Ma Y. Positive association between Interleukin-8 −251A>T polymorphism and susceptibility to gastric carcinogenesis: a meta-analysis. Cancer Cell Int. 2013;13:100.
68. Smith SM. Role of Toll-like receptors in *Helicobacter pylori* infection and immunity. World J Gastrointest Pathophysiol. 2014;5:133–46.
69. Varga MG, Peek RM. DNA transfer and Toll-like receptor modulation by *Helicobacter pylori*. Curr Top Microbiol Immunol. 2017;400:169–93.
70. Seya T, Shime H, Ebihara T, Oshiumi H, Matsumoto M. Pattern recognition receptors of innate immunity and their application to tumor immunotherapy. Cancer Sci. 2010;101:313–20.
71. Su B, Ceponis PJ, Lebel S, Huynh H, Sherman PM. *Helicobacter pylori* activates toll-like receptor 4

expression in gastrointestinal epithelial cells. Infect Immun. 2003;71:3496–502.

72. Pimentel-Nunes P, Afonso L, Lopes P, Roncon-Albuquerque R Jr, Gonçalves N, Henrique R, et al. Increased expression of toll-like receptors (TLR) 2, 4, 5 in gastric dysplasia. Pathol Oncol Res. 2011;17:677–83.
73. Castaño-Rodríguez N, Kaakoush NO, Goh KL, Fock KM, Mitchell HM. The role of TLR2, TLR4 and CD14 genetic polymorphisms in gastric carcinogenesis: a case-control study and meta analysis. PLoS One. 2013;8:e60327.
74. Bagheri N, Azadegan-Dehkordi F, Sanei H, Taghikhani A, Rahimian G, Salimzadeh L, et al. Association of TLR4 single-nucleotide polymorphism with *H. pylori* associated gastric diseases in Iranian patients. Clin Res Hepatol Gastroenterol. 2014;38:366–71.
75. Zhou Q, Wang C, Wang X, Wu X, Zhu Z, Liu B, Su L. Association between TLR4 (+896A/G and +1196C/T) polymorphisms and gastric cancer risk: an updated meta-analysis. PLoS One. 2014;9:e109605.
76. Hold GL, Rabkin CS, Chow WH, Smith MG, Gammon MD, Risch HA, et al. A functional polymorphism of toll-like receptor 4 gene increases risk of gastric carcinoma and its precursors. Gastroenterology. 2007;132:905–12.
77. Santini D, Angeletti S, Ruzzo A, Dicuonzo G, Galluzzo S, Vincenzi B, et al. Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms in gastric cancer of intestinal and diffuse histotypes. Clin Exp Immunol. 2008;154:360–4.
78. Zhao X, Kang S, Liu L, Zhang D. Correlation of Asp299Gly and Thr399Ile polymorphisms in toll-like receptor 4 gene with digestive cancer risk: a meta-analysis. Biomed Rep. 2013;1:294–302.
79. Yuan M, Xia J, Ma L, Xiao B, Yang Q. Lack of the toll-like receptor 4 gene polymorphisms Asp299Gly and Thr399ile in a Chinese population. Int J Neurosci. 2010;120:415–20.
80. Tahara T, Arisawa T, Shibata T, Hirata I, Nakano H. Absence of common polymorphisms of toll like receptor 4 (TLR4): Asp299Gly, Thr399Ile in patients with gastroduodenal diseases in Japan. J Clin Biochem Nutr. 2007;40:62–5.
81. Kim YS, Hwang YJ, Kim SY, Yang SM, Lee KY, Park IB. Rarity of TLR4 Asp299Gly and Thr399Ile polymorphisms in the Korean population. Yonsei Med J. 2008;49:58–62.
82. Wang J, Guo X, Yu S, Song J, Zhang J, Cao Z, et al. Association between CD14 gene polymorphisms and cancer risk: a meta analysis. PLoS One. 2014;9:e100122.
83. Mukherjee T, Hovingh ES, Foerster EG, Abdel-Nour M, Philpott DJ, Girardin SE. NOD1 and NOD2 in inflammation, immunity and disease. Arch Biochem Biophys. 2019;670:69–81.
84. Mommersteeg MC, Yu J, Peppelenbosch MP, Fuhler GM. Genetic host factors in *Helicobacter pylori*-induced carcinogenesis: emerging new paradigms. Biochim Biophys Acta Rev Cancer. 2018;1869:42–52.
85. Lees CW, Barrett JC, Parkes M, Satsangi J. New IBD genetics: common pathways with other diseases. Gut. 2011;60:1739–53.

Part III

Diagnosis

8

Serology

Nayoung Kim

Abstract

Serological diagnosis is noninvasive and relatively economical and has less possibility of showing false-negative results in the situations of hemorrhagic ulcer, taking medications like antibiotics or proton pump inhibitors. In addition, it performs mass epidemiological surveys simultaneously. However, it is not recommended to monitor changes after antimicrobial treatment because this diagnosis hardly differentiates past *Helicobacter pylori* (*H. pylori*) infections and current active infections. There are various types of serological diagnoses of *H. pylori* existence such as bacterial agglutination, complement fixation, indirect immunofluorescence test (IIF), enzyme immunoassay (EIA), and enzyme-linked immunosorbent assay (ELISA). Among those, ELISA is the most popular method in the world.

Keywords

Helicobacter pylori · Serology · Diagnosis
ELISA

8.1 Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative, spiral bacillus that the half of entire global population is suspected to be its carrier. There has been much research on *H. pylori* since the 1980s because this bacterium has been remarked as a main reason for gastritis, peptic ulcer, and stomach cancer. Especially, the International Agency of Research on Cancer (IRAC) of WHO classifies *H. pylori* as a primary carcinogenic factor of gastric cancer (GC) [1], and there are many laboratory results that support this categorization. Thus, *H. pylori* infection diagnosis is important. There are invasive diagnoses, such as rapid urease test and polymerase chain reaction (PCR), that require endoscopy and noninvasive diagnoses, such as serology and urea breath test (UBT), that do not need an endoscopy. Among those diagnoses, serological diagnosis is used widely because it is noninvasive, relatively economical, and can be simultaneously applied to many patients for epidemiological surveys [2]. This chapter will discuss the advantages and the disadvantages of serological diagnoses, with an emphasis on the classification of the serological test, and its sensitivity and specificity for the diagnosis of *H. pylori* infection in the world.

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8.2 Advantages and Disadvantages of Serological Diagnosis

Serological diagnosis can be a useful way to detect *H. pylori* preliminary infection because it is easy, fast, noninvasive, and relatively economical and tends to show less false-negative results under certain situations, such as taking antibiotics or hemorrhagic ulcer condition, compared with other diagnoses. However, the serological diagnosis could show false-positive results for several months or years on samples because it hardly differentiates the current active infection from the previous infection, which has been treated and no more infection is shown [3]. Thus, the serological diagnosis is not recommended after antimicrobial treatment because an average of 1 year is necessary until either antibody is undetectable or antibody titer is reduced to 50% after antimicrobial treatment [3]. Rather, it is utilized as a preliminary, selective diagnosis for *H. pylori* infection, not after the antimicrobial treatment [3, 4]. For instance, the serological diagnosis is used for epidemiological surveys, not as a diagnosis before or after antimicrobial treatment, in China [5]. On the contrary, the diagnosis is recommended to be performed before or after antimicrobial treatment in Japan; *H. pylori* eradication is considered to be successful if serological antibody titer that is performed from 6 to 12 months after antimicrobial treatment is 50% less than the titer that is performed before the treatment [5].

8.3 Serological Diagnosis

There are numerous serological diagnoses, such as bacterial agglutination, complement fixation, indirect immunofluorescence test (IIF), enzyme immunoassay (EIA), and enzyme-linked immunosorbent assay (ELISA), and ELISA is the most prevalent diagnosis [4, 6]. This section will introduce these test methods, diagnostic kits, and the sensitivity and specificity of each diagnosis.

8.3.1 Bacterial Agglutination, Complement Fixation, and Indirect Immunofluorescence Test (IIF)

Agglutination is a method when a particle with certain size, such as erythrocyte, is merged with a suspension-form antibody that is made by the absorption of antigens of fine particles like kaolin and latex and forms an immune complex. This method is used for either blood type determination or bacterial identification. Agglutination kits, which utilize an agglutination reagent that binds to *H. pylori* antibodies in serum, are Hemkit® (LD-Diagnostika, Heiden, FRG), Pyloriset Dry (Orion Diagnostica, Espoo, Finland), and so on. The sensitivity and specificity of these agglutination diagnoses are reported to be 71%–79% and 81%–82%, respectively. Also, the *H. pylori* strain status of this diagnosis was determined by one of three methods: histological analysis, ^{13}C-urea breath test (^{13}C-UBT), and rapid urease test [7, 8] (Table 8.1).

Next, complement system-related antigen–antibody reactions are cytolytic reactions and complement fixation reactions; the latter method is used for the bacterial identification and serological diagnosis of *H. pylori* infection. Specifically, a complement system is added to a mixture that contains immune complexes due to the antigen–antibody reaction. The complement system will be absorbed and metabolized if the immune complex exists in the mixture. Then, the antigen–antibody reaction can be analyzed by measuring the amount of reduced complement system compared with the amount of the added system at the beginning. According to the previous research, the sensitivity and specificity of the complement fixation test (CFT) for *H. pylori* (Institute Virion, Rüschlikon, Switzerland) were 86.6% and 77.6%, respectively, compared with stomach biopsy [9] (Table 8.2). In addition, another research reported that the sensitivity and the specificity of CFT reagents were 71% and 90%, respectively, compared with the Giemsa stain [10] (Table 8.2).

Table 8.1 Hemagglutination tests for the detection of *H. pylori*

Product name	Sensitivity (%)	Specificity (%)	Diagnostic reference test	Reference
Pyloriset dry	91.1	87.5	Rapid urease test	[7]
			^{13}C-UBT, histology	
Hemki®	71–79	81–82		[8]

UBT urea breath test

Table 8.2 Complement fixation tests (CFT) for the detection of *H. pylori*

Product name	Sensitivity (%)	Specificity (%)	Diagnostic reference test	Reference
CFT *H. pylori*	86.6	77.6	Histology	[9]
CFT *H. pylori*	71.0	90.0	Culture and histology	[10]

Table 8.3 Indirect immunofluorescence test (IIF) for the detection of *H. pylori*

Product name	Sensitivity (%)	Specificity (%)	Diagnostic reference test	Reference
Immunfluoreszenz IgG test	62.8	74.4	Histology	[9]
IIF	95.9	88.8	Culture, rapid urease test, and stain with carbolfuchsin	[11]

Third, a labeled antibody method is defined as attaching a label on an immunoglobulin for performing the antigen–antibody reaction, to find antigen existence and to measure the amount of antigen. IIF, EIA, and ELISA belong to the labeled antibody method.

The immunofluorescence test utilizes fluorescent pigments, such as fluorescein isothiocyanate. The direct immunofluorescence test is a method where a fluorescent substance-attached antibody solution reacts with a slide and then it is washed off, and IIF is a technique that a slide reacts with an antibody solution and then reacts with the fluorescent substance-attached anti-immunoglobulin antibody to diagnose a result. When the slide is passed through ultraviolet (UV) light, the fluorescent antibody-attached region will be shown as fluorescent green, and the rest of the region will be shown as dark. For instance, sensitivity and specificity of the Immunfluoreszenz IgG Test (Bios, *Graefelfing, Germany*) were 62.8% and 74.4%, respectively, compared with stomach biopsy [9] (Table 8.3). Also, another research reported that the sensitivity and specificity of IIF were 95.9% and 88.8%, respectively, compared with culture, rapid urease test, and smears stained (carbolfuchsin) [11] (Table 8.3).

8.3.2 EIA and ELISA

EIA and ELISA are immunoserological methods that measure either antigen or antibody; enzyme-linked antigen or antibody reacts with a sample to form an antigen–antibody–enzyme complex. Then, a substrate is added to this complex to induce colorization, to measure the degree of color change to extract and to quantify either antigen or antibody. Color change can be measured by absorbance, fluorescence substance, or radioactive label, and all of these techniques belong to EIA. For ELISA, the antigen–antibody–enzyme complex is mixed with a substrate to induce coloration and then the color change is measured only with absorbance. ELISA is also a type of EIA. Commercialized EIAs are PYloriset®-IgG EIA (Orion Diagnostica, Espoo, Finland), Immulite® 2000 *H. pylori* IgG system (Diagnostic Products, Los Angeles, CA), VIDAS® *H. pylori* IgG (BioMérieux, Marcy-l'Étoile, France), Radim® *H. pylori*-EIA-Well (Radim®, Rome, Italy), and so on. Sensitivities of EIA-based tests are reported to be 68.0–100% and 79.3–97.0%; criteria of *H. pylori* diagnosis of each research are depicted in Table 8.4 [12–16]. Genedia® *H. pylori* (Green Cross Co., Seoul, South Korea), GAP® Test IgG

Table 8.4 Enzyme immunoassay (EIA) for the detection of *H. pylori*

Product name	Sensitivity (%)	Specificity (%)	Diagnostic reference test	Reference
Pyloriset®-IgG	82.4–92.0	71.4–84.0	Culture and histology	[12]
			Rapid urease test histology or ^{13}C-UBT	[13]
Immulite®	91.0	100	Histology, rapid urease test	[14]
VIDAS®	100	0	Rapid urease test, histology	[13]
Radim®	88.0–95.6	93.8–97.8	Culture, histology, rapid urease test	[15]
			Genedia®	[16]

UBT urea breath test

Table 8.5 Enzyme-linked immunosorbent assay (ELISA) for the detection of *H. pylori*

	Enzyme-linked immunosorbent assay (ELISA)			
Product name	Sensitivity (%)	Specificity (%)	Detection of *H. pylori* presence	Reference
Genedia®	93.2–100	81.3–92	Culture, histology, rapid urease test	[15, 16]
			Rapid urease test	[17]
			Histology or ^{13}C-UBT	
GAP®	60.0–93.8	79.3–87.5	Culture, histology, rapid urease test	[15]
			Rapid urease test	[17]
			Histology or ^{13}C-UBT	

UBT urea breath test

(Bio-Rad, Milan, Italy), and Chorus *Helicobacter* IgG (DIESSE Diagnostica Senese, Siena, Italy) belonged to the ELISA test, and their sensitivities and specificities were reported to be 93.2%–100% and 60.0%–97.0%, respectively, as given in Table 8.5 [15–17].

8.3.3 Commercial Serological ELISA Kits Depending on *H. pylori* Antigen

When a comparison of 17 commercial ELISA serological kits was carried out in France, two to four of the ELISAs presented an excellent performance [2, 18]. When a line assay using six recombinant proteins corresponding to virulence factors (CagA, VacA, GroEL, gGT, HcpC, and UreA) was validated on a group of 600 patients (42% *H. pylori* positive by histology) in Germany, it showed 97.6% sensitivity and 96.2% specificity, which is an improvement on the currently available serological tests [19]. The same group in collaboration with researchers in Iran was able to identify an *H. pylori* protein, FliD, essential in the assembly of the flagella [2]. The recombinant FliD protein was tested on a group of 618 patients (51.4% *H. pylori* positive) with 97.4% sensitivity and 99% specificity using a line assay and 97% and 96% by ELISA, respectively [20]. Other attempts to select antigenic proteins of potential diagnostic value were made (CafI, UreG, UreB), but have not been evaluated yet [21]. Interestingly, using Helicoblot 2.1 (Genelabs Diagnostics, Singapore), it was possible to identify a low molecular weight protein (35 kDa) associated with a low risk of GC (odds ratio (OR) 0.4; 95% confidence interval (CI), 0.1–0.9) and the VacA protein associated with a high risk of GC (OR 2.7; 95% CI, 1–7.1) among patients with GC (n = 102) and dyspepsia (n = 122) in Iran [22].

8.3.4 Genedia® *H. pylori* ELISA and Its Use on Nationwide *H. pylori* Epidemiological Survey in Korea

Genedia® *H. pylori* kit, which was made by Green Cross Co., is an ELISA reagent [17]. This reagent was made of ultrasound-treated antigens that are from MBRIHP 2 of Korean chronic gastritis patient's strains and from MBRIHP 8 Korean duodenal ulcer patient's strains, and it helps to extract anti-*H. pylori* IgG antibody in the patient's serum. This measurement was performed at the room temperature; a sample or a reference drug reacts with an antigen-absorbed plate; the plate is washed off and a dilution-concentration adhesive is added and washed again under 37 ± 1 °C and then a substrate is added to react again. Absorbance is measured at 450 nm; if a sample's absorbance value is higher, then the cutoff value is considered positive, and negative if the absorbance value is lower than the cutoff value. In 1998, Kim et al. performed a bacterial culture and rapid urease test and reported the sensitivity and specificity of Genedia® as 97.8% and 92%, respectively [23]. However, Yang et al. compared histological analysis (H&E, Giemsa stain) to Genedia® to report sensitivity, specificity, positive predictive value, and negative predictive value of Genedia® as 96.2%, 56.8%, 78.9%, and 90.0%, respectively [24], which are rather low values. The difference of sensitivity and specificity between researchers probably depends on which test has been referenced. The nationwide mass epidemiological survey of *H. pylori*-positive rate using this Genedia® kit was performed in 1999 [25], 2005 [16], 2011 [26], and 2016–2017 [27] in Korea, and the results showed a decrease of *H. pylori* positive as the socioeconomic status was improved.

8.3.5 Genedia® *H. pylori* ELISA and Its Use on Nationwide *H. pylori* Epidemiological Survey in Korea

Immulite®, a solid-phase, two-step chemiluminescent enzyme immunoassay, is commonly used, easy to perform, inexpensive, and widely available for the diagnosis of *H. pylori*. This kit was used in the nationwide mass epidemiological survey of *H. pylori*-positive rate in 2016–2017 in Korea in addition to the Genedia® kit [27]. After the publication of the survey, we examined the performance of Immulite® compared to Genedia® in 300 Korean health check-up subjects [28]. There were significant correlation (Pearson coefficient (r) = 0.903, $p < 0.0001$) and almost perfect agreement (Cohen's Kappa coefficient (κ) = 0.987, $p < 0.0001$) between the results of Genedia® and Immulite®. In addition, two serologic tests were compared in predicting atrophic gastritis (AG) or intestinal metaplasia (IM) on endoscopy [28]. The area under the ROC curve (AUC) for AG using Genedia® and Immulite® were 0.590 and 0.604 and showed no different statistical difference in prediction about AG (Z-statistics = −0.517, $p = 0.605$) [28] (Fig. 8.1a).

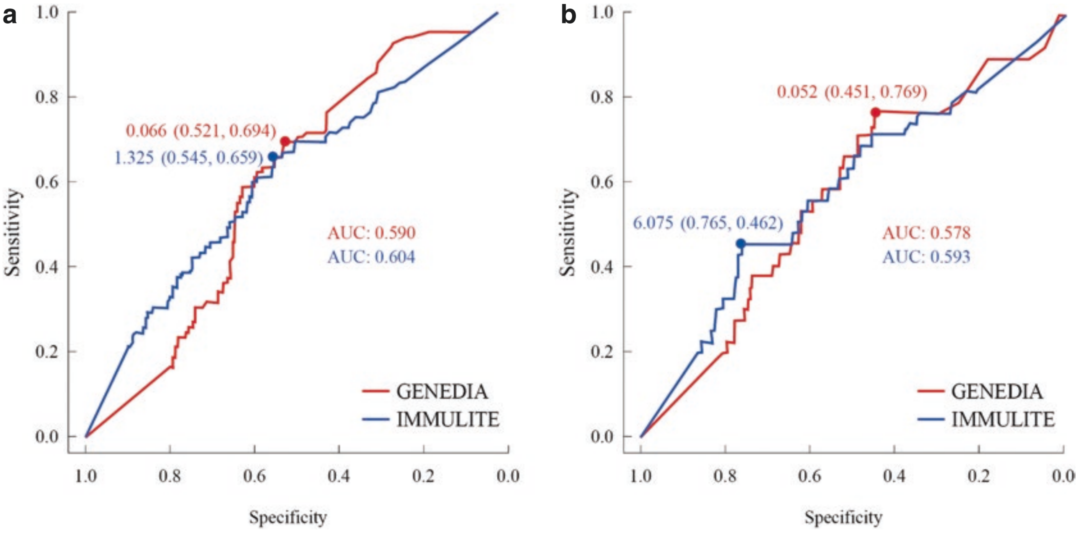

Fig. 8.1 Receiver operator characteristic curve in the diagnosis of endoscopic atrophic gastritis (**a**) or endoscopic intestinal metaplasia (**b**) by anti-*H. pylori* immunoglobulin (Ig)G by Genedia® and Immulite®. (**a**) The optimal cut-off values of serum anti-*H. pylori* IgG for predicting endoscopic atrophic gastritis are 0.066 on Genedia® and 1.325 on Immulite®. (**b**) The optimal cut-off values of serum anti-*H. pylori* IgG for predicting endoscopic intestinal metaplasia are 0.052 on Genedia® and 6.075 on Immulite® (Adapted from Lim et al. [28])

The AUC for IM by Genedia® and Immulite® was 0.578 and 0.593, and there was no statistical difference in prediction about IM between the two values (*Z*-statistics = −0.398, $p = 0.691$) [28] (Fig. 8.1b). There was no significant difference in the diagnosis of *H. pylori* infection between Genedia® and Immulite®.

8.4 Conclusions

Serological diagnosis is noninvasive, is relatively economical, has less possibility of showing false-negative results in the situation of hemorrhagic ulcer or taking medications like antibiotics or proton pump inhibitor, and can perform a mass epidemiological survey at once. However, it is not recommended to monitor change after antimicrobial treatment because it hardly differentiates past *H. pylori* infections and current active infections [29, 30]. The ELISA method is the most prevalent method in the world. There are many kinds of serological kits with various results of sensitivity and specificity depending on their antigenic proteins of potential diagnostic value. Thus, it is necessary to validate its efficacy and usefulness in each country.

References

1. International Agency for Research on Cancer, World Health Organization. Schistosomes, liver flukes, and *Helicobacter pylori*. IARC monographs on the evaluation of carcinogenic risks to humans. Lyon: IARC; 1994;61. p. 1–241.
2. Megraud F, Bessede E, Lehours P. Diagnosis of *Helicobacter pylori* infection. Helicobacter. 2014;19 (Suppl 1):6–10.
3. Lee JH, Kim N, Chung JI, Kang KP, Lee SH, Park YS, et al. Long-term follow-up of *Helicobacter pylori* IgG serology after eradication and reinfection rate of *H. pylori* in South Korea. Helicobacter. 2008;13:288–94.
4. Kim SG, Jung HK, Lee HL, Jang JY, Lee H, Kim CG, et al. Guidelines for the diagnosis and treatment of *Helicobacter pylori* infection in Korea, 2013 revised edition. J Gastroenterol Hepatol. 2014;29:1371–86.
5. Lee SY. Current progress toward eradicating *Helicobacter pylori* in East Asian countries: differences in the 2013 revised guidelines between China, Japan, and South Korea. World J Gastroenterol. 2014;20:1493–502.
6. Choi YJ, Kim N, Lim J, Jo SY, Shin CM, Lee HS, et al. Accuracy of diagnostic tests for *Helicobacter pylori* in patients with peptic ulcer bleeding. Helicobacter. 2012;17:77–85.
7. Lozniewski A, De Korwin JD, Conroy MC, Plenat F, Weber M. Evaluation of pyloriset dry, a new rapid agglutination test for *Helicobacter pylori* antibody detection. J Clin Microbiol. 1996;34:1773–5.
8. Nilius M, Wex T, Muller-Dietz G, Leodolter A, Schilling D, Malfertheiner P. Comparative evaluation of two hemagglutination tests for the detection of anti-*Helicobacter pylori* antibodies. Diagn Microbiol Infect Dis. 2001;41:221–3.
9. Allerberger F, Oberhuber G, Wrba F, Puspok A, Dejaco C, Dierich MP. Detection of *Helicobacter pylori* infection using single serum specimens: comparison of five commercial serological tests. Hepatogastroenterology. 1996;43:1656–9.
10. Goossens H, Glupczynski Y, Burette A, Van den Borre C, DePrez C, Bodenmann J, et al. Evaluation of a commercially available complement fixation test for diagnosis of *Helicobacter pylori* infection and for follow-up after antimicrobial therapy. J Clin Microbiol. 1992;30:3230–3.
11. Rocha GA, Queiroz DM, Mendes EN, Oliveira AM, Moura SB, Barbosa MT, et al. Indirect immunofluorescence determination of the frequency of anti-*H. pylori* antibodies in Brazilian blood donors. Braz J Med Biol Res. 1992;25:683–9.
12. Granberg C, Mansikka A, Lehtonen OP, Kujari H, Grönfors R, Nurmi H, et al. Diagnosis of *Helicobacter pylori* infection by using pyloriset EIA-G and EIA-A for detection of serum immunoglobulin G (IgG) and IgA antibodies. J Clin Microbiol. 1993;31:1450–3.
13. Naumov I, Fenjvesi A. Correlation between rapid urease test and pathohistological gastrobiopsy finding with positive immunological test in detecting *Helicobacter pylori* infection. Med Pregl. 2011;64:413–7.
14. van Der Ende A, van Der Hulst RW, Roorda P, Tytgat GN, Dankert J. Evaluation of three commercial serological tests with different methodologies to assess *Helicobacter pylori* infection. J Clin Microbiol. 1999;37:4150–2.
15. Eom HS, Kim PS, Lee JW, Pai SH, Lee JW, Choi W, et al. Evaluation of four commercial enzyme immunoassays for detection of *Helicobacter pylori* infection. Korean J Gastroenterol. 2001;37:312–8.
16. Yim JY, Kim N, Choi SH, Kim YS, Cho KR, Kim SS, et al. Seroprevalence of *Helicobacter pylori* in South Korea. Helicobacter. 2007;12:333–40.
17. Chung IS, Kim SW, Go JS, Kim NY, Kim JG, Kim JH, et al. Accuracy of Genedia™ *H. pylori* ELISA for the diagnosis of *Helicobacter pylori* infection in Korean population. Korean J Med. 2001;61:17–23.
18. Linz B, Windsor HM, Gajewski JP, Hake CM, Drautz DI, Schuster SC, et al. *Helicobacter pylori* genomic microevolution during naturally occurring transmission between adults. PLoS One. 2013;8:e82187.

19. Krebes J, Morgan RD, Bunk B, Spröer C, Luong K, Parusel R, et al. The complex methylome of the human gastric pathogen *Helicobacter pylori*. Nucleic Acids Res. 2014;42:2415–32.
20. Furuta Y, Namba-Fukuyo H, Shibata TF, Nishiyama T, Shigenobu S, Suzuki Y, et al. Methylome diversification through changes in DNA methyltransferase sequence specificity. PLoS Genet. 2014;10:e1004272.
21. Gilbreath JJ, Pich OQ, Benoit SL, Besold AN, Cha JH, Maier RJ, et al. Random and site specific mutagenesis of the *Helicobacter pylori* ferric uptake regulator provides insight into Fur structure-function relationships. Mol Microbiol. 2013;89:304–23.
22. Correia M, Casal S, Vinagre J, Seruca R, Figueiredo C, Touati E, et al. *Helicobacter pylori*'s cholesterol uptake impacts resistance to docosahexaenoic acid. Int J Med Microbiol. 2013;304:314–20.
23. Kim SY, Ahn JS, Ha YJ, Doh HJ, Jang MH, Chung SI, et al. Serodiagnosis of *Helicobacter pylori* infection in Korean patients using enzyme-linked immunosorbent assay. J Immunoass. 1998;19:251–70.
24. Yang JH, Lim YA, Lee KB. Evaluation of diagnostic usefulness for Genedia® *Helicobacter pylori* ELISA. J Clin Pathol Qual Control. 2000;22:197–201.
25. Kim JH, Kim HY, Kim NY, Kim SW, Kim JG, Kim JJ, et al. Seroepidemiological study of *Helicobacter pylori* infection in asymptomatic people in South Korea. J Gastroenterol Hepatol. 2001;16:969–75.
26. Lim SH, Kwon JW, Kim N, Kim GH, Kang JM, Park MJ, et al. Prevalence and risk factors of *Helicobacter pylori* infection in Korea; nationwide multicenter study over 13 years. BMC Gastroenterol. 2013;13:104.
27. Lim SH, Kim N, Kwon JW, Kim SE, Baik GH, Lee JY, et al. Trends in the seroprevalence of *Helicobacter pylori* infection and its putative eradication rate over 18 years in Korea: a cross-sectional nationwide multicenter study. PLoS One. 2018;13:e0204762.
28. Lim SH, Kim N, Kim SE, Baik GH, Lee JY, Park KS, et al. A comparison of accuracy between IMMULITE2000® and GENEDIA® for *Helicobacter pylori* infection. Korean J Helicobacter Up Gastrointest Res. 2020;20:54–62.
29. Godbole G, Francis Mégraud F, Emilie Bessède E. Diagnosis of *Helicobacter pylori* infection. Helicobacter. 2020;25 (Suppl 1):e12735.
30. Wang YK, Kuo FC, Liu CJ, Wu MC, Shih HY, Wang SSW, et al. Diagnosis of *Helicobacter pylori* infection: current options and developments. World J Gastroenterol. 2015;21:11221–35.

9

Histopathologic Diagnosis of *H. pylori* Infection and Associated Gastric Diseases

Hye Seung Lee

Abstract

Helicobacter pylori (*H. pylori*) can be diagnosed by histopathologic examination, which is considered to be a standard method, since not only it observes *H. pylori* directly but also it evaluates the degree of gastric inflammation, gastritis-related intestinal metaplasia (IM), atrophic gastritis (AG), and tumor. Among various staining methods, hematoxylin and eosin (H&E) stain is a routine procedure in daily practice. However, if H&E stain is not enough for histological diagnosis, ancillary tests including special staining and immunohistochemistry (IHC) are needed for accurate diagnosis. Among various ancillary tests, Giemsa stain has high sensitivity, and silver stain and IHC have high sensitivity and specificity. Recently, several molecular techniques have been applied in the diagnosis of *H. pylori* or *H. pylori* resistant to antibiotics. The initial stage of *H. pylori* infection is mostly asymptomatic, but it can develop into acute and chronic gastritis. *H. pylori*-related chronic gastritis shows not only lymphocyte and plasma cell infiltration but also neutrophil infiltration inside the epithelium. *H. pylori*-related chronic gastritis can lead to sequelae, such as AG, IM, and lymphoid follicles. Moreover, *H. pylori* infection is a cause of mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer, and especially, the infection has a close relationship with extranodal marginal zone B lymphoma of MALT and intestinal-type gastric adenocarcinoma. Therefore, histopathologic diagnosis is necessary for gastritis patients, and it should include the degree of gastritis and the presence of *H. pylori*, sequela of gastritis, and malignant tumor.

Keywords

Gastric cancer · Gastritis · *Helicobacter pylori* · Histology · Pathology

9.1 Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative bacteria that belongs to the *Bacillus* genus. Diseases like acute and chronic gastritis, gastric ulcer, duodenal ulcer, and gastric cancer (GC) (gastric adenocarcinoma and malignant lymphoma) are caused by *H. pylori*, and the WHO has classified this bacteria as a definite or group I human carcinogen in 1994 [1, 2]. Either gastritis or gastric ulcer can be caused by nonsteroidal anti-inflammatory drug (NSAID), autoimmunity, infection other than *H. pylori*, and so on. Thus, *H. pylori* detection and clinical findings are important for accurate diagnosis and optimal treatment. This chapter is going to introduce

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histopathologic features of gastric diseases associated with *H. pylori* infection.

9.2 Histological Diagnosis of *H. Pylori*

H. pylori diagnosis is divided into invasive testing that requires endoscopy and noninvasive testing that does not require endoscopy [3, 4]. Among diagnostic methods, histological examination that uses endoscopic biopsy samples is considered to be a standard method because not only it observes *H. pylori* directly but also it evaluates the degree of gastric inflammation, gastritis-related intestinal metaplasia (IM), atrophic gastritis (AG), and tumor.

H. pylori is a spiral or curved bacteria that has a length of 2–4 μm. It has flagella mainly at one side, but sometimes flagella can be seen on both sides. *H. pylori* can be observed on the thick mucus of the surface of the gastric mucosa via optical microscope under high magnification. These bacteria cannot be seen on the surface of the erosion or ulcer but can be seen on the surrounding mucosa of the erosion or ulcer region. It is also hard to observe in IM and AG regions.

9.2.1 Hematoxylin and Eosin (H&E) Stain

Hematoxylin and eosin (H&E) stain is routinely used for pathological diagnosis in daily practice, so additional stain is not necessary and can directly observe bacteria under high magnification (Fig. 9.1a). The sensitivity and specificity of *H. pylori* diagnosis with H&E stain has been reported as 69–93% and 87–90%, respectively [5, 6]. The sensitivity can be different based on the types of reagent, the intensity of the stain, and the concentration of bacteria in a sample. Especially, a sample with a low number of *H. pylori* leads to low sensitivity due to the difficulty of bacterial observation, and pathologists' experience and skill also affect the accuracy of *H. pylori* diagnosis [7]. Thus, if *H. pylori* is not found in a histologically gastritis-positive sample, additional special stain or immunohistochemistry (IHC) is recommended for precise determination [8]. However, only a histopathologic diagnosis is enough, if *H. pylori* is clearly found and proper corresponding inflammatory features are also observed.

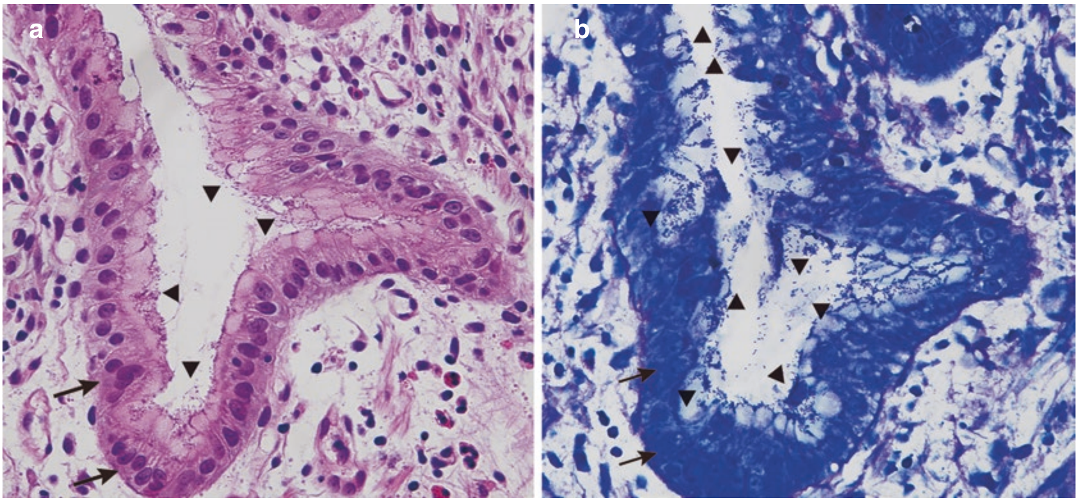

Fig. 9.1 Histopathologic findings of *H. pylori*. (**a**) Hematoxylin–eosin (H&E) staining. The morphological feature of epithelial and stromal cells is well preserved, but the sensitivity of the bacteria is low (*arrowhead*: *H. pylori*; *arrow*: nucleus of the epithelial cell). (**b**) Giemsa staining. It has higher bacterial sensitivity than H&E staining, but it is difficult to recognize the morphology of epithelial and stromal cells (*arrowhead*: *H. pylori*; *arrow*: nucleus of the epithelial cell)

9.2.2 Special Stain and Immunohistochemistry (IHC)

Ancillary tests to diagnose *H. pylori* infection can be categorized into silver stain, special stains other than silver stain, and IHC. First of all, examples of silver stains are Warthin–Starry stain, modified Steiner stain, El-Zimaity stain, and Genta stain [9, 10]. An advantage of silver stain is that it shows high sensitivity and specificity like IHC but its disadvantages are its high price and inconsistent staining results due to blurry background caused by nonspecific stain [11].

Second, special stains other than silver stain are Diff-Quick stain, crystal violet stain, Giemsa stain, and so on [7, 12]. To begin with, Giemsa stain is easy, quick, and cheap and shows relatively consistent stains, so it is preferred by many pathology departments (Fig. 9.1b). Giemsa stain can better differentiate *H. pylori* from the mucous material on the mucosal surface [13], but this stain can show a false-positive result [14]. Next, IHC has higher accuracy in terms of diagnostic results than special stains because IHC has less possibility of showing false-positive results [15]. Both polyclonal and monoclonal antibodies are commercially available. IHC is helpful to differentiate *H. pylori* from other microorganisms, especially when a patient has AG or takes a proton pump inhibitor (PPI). Moreover, IHC is helpful in diagnosing when *H. pylori* shows a nonselective form, not a spiral form; *H. pylori* can be in coccoid form, and then, it is difficult to distinguish from other coccoid-form bacteria. There is a controversy on the toxicity and survival ability of the coccoid form of *H. pylori*, thus reporting it as a coccoid form in pathologic diagnosis is recommended [16]. Nonetheless, it will be hard to observe the bacteria even with IHC, if there is a really small number of *H. pylori*. In addition, IHC can show a positive result on different *H. pylori* species (*H. heilmannii*) if polyclonal antibody is utilized [17].

Histological staining methods for *H. pylori* diagnosis are summarized in Table 9.1. A single perfect stain for *H. pylori* diagnosis does not exist; rather, a proper stain method will be selected by each pathologist or pathology department. In addition, sensitivity and specificity can be increased, if histological features, such as degree of inflammation and morphologic feature and size of spiral bacteria, are considered at the diagnosis. However, the site, number, and size of gastric biopsy can also lead to false-negative results on *H. pylori* histological diagnosis, so these factors should be considered when a false negative is suspected.

Table 9.1 Comparison of various staining methods for detecting *H. pylori*

Stain	Sensitivity	Specificity	Stain method	Cost	Note
H&E	Low	Low	Easy	Low	Other staining is necessary if the false negative is suspected
Warthin–starry	High	High	Difficult	High	Background stains can be blurry, and H&E is necessary for diagnosis
El-Zimaity	High	High	Difficult	Intermediate	Efficient if low bacterial concentration
Genta	High	High	Difficult	High	Difficult method and takes a long time
Diff-quick/ crystal violet	Intermediate	Intermediate	Easy	Low	H&E is necessary for diagnosis
Giemsa	Intermediate	Low	Easy	Low	H&E is necessary for diagnosis
Immunostaining	High	Very high	Difficult	High	Staining takes a long time, and H&E is necessary for diagnosis

H&E Hematoxylin–eosin staining

9.3 Molecular Tests

In addition to direct observation of micro-organisms by H&E or other special staining, *H. pylori* infection can be diagnosed by molecular techniques. Molecular techniques have been developed for detecting specific regions of *H. pylori* genomic DNA in gastric biopsy specimens. Various bacterial genes, such as 16S rRNA, 23S rRNA, *cagA*, *flaA*, *vacA,* and *ure* genes, have been candidates for the detection [13]. Technically, polymerase chain reaction (PCR), real-time PCR, droplet digital PCR (ddPCR), fluorescence in-situ hybridization (FISH), and next-generation sequencing (NGS) were studied, and several techniques are used in daily practice [18]. These molecular methods have the advantage of not only diagnosing *H. pylori* but also detecting *H. pylori* resistant to some antibiotics. Among various methods, PCR-based techniques are simple, fast, and less expensive, thus easily used in daily practice.

A few mutations in the peptidyltransferase loop of the 23S rRNA gene have been reported to be associated with clarithromycin resistance (A2142C, A2142G, and A2143G) [19]. Dual-priming oligonucleotide (DPO)-based multiplex PCR has been developed to specifically detect *H. pylori* resistant to clarithromycin [20]. The positive rate of DPO-based multiplex PCR for *H. pylori* detection (41.5%) was slightly lower than that of histology (42.5%) but higher than culture (22.2%). The concordance rate of clarithromycin susceptibility between DPO-based multiplex PCR and culture was 94.1%. Lehours et al. also reported that clarithromycin susceptibility results by DPO-based multiplex PCR were concordant with those by culture in 95.3% of gastric biopsies [21].

The PNA probe-based real-time PCR method with fluorescence melting peak signals was developed for detecting *H. pylori* and clarithromycin resistance [22]. By this method, the *H. pylori*-positive rate was 95.6% (259/271) of *H. pylori*-positive cases by IHC, and the A2142G/A2143G mutation detection sensitivity was 100%. Noh et al. also showed that 68 of 70 (97.1%) specimens with confirmed *H. pylori* infection were positive for *H. pylori* by this real-time PCR method, and clarithromycin resistance was observed in 11 of 13 (84.6%) specimens with confirmed clarithromycin resistance [23].

ddPCR, one of the recently developed methods, was also used to detect *H. pylori* or clarithromycin resistance. By ddPCR methods, low-density infection could be detected in 36% of *H. pylori*-negative gastric biopsies, and furthermore, the density of infection could be quantified [24]. Clarithromycin resistance could also be examined by ddPCR with high concordance with culture test [25], but ddPCR needs additional equipment and is more expensive than other PCR-based tests.

In addition to PCR-based methods, FISH and NGS were tried to detect *H. pylori* or clarithromycin resistance. FISH has the advantages of being able to know the spatial distribution of *H. pylori* and to visualize the co-existence of *H. pylori* resistant and susceptible to antibiotics within one tissue sample [26]. However, the FISH method is difficult and requires a dark room and an experienced pathologist. Recently, comprehensive information on whole micro-organisms in gastric biopsies can be obtained by the NGS method. NGS is expensive and labor-intensive; thus, it is not used in pathology laboratories for *H. pylori* detection in gastric biopsies. In addition, NGS cannot differentiate between live, coccoid, and dead cells like other DNA-based methods [18].

9.4 Pathologic Features of *H. Pylori*-Associated Gastritis

9.4.1 Acute Gastritis

The initial stage of *H. pylori* infection is mostly asymptomatic. Edema and mild neutrophilic infiltration in lamina propria are observed, like other acute infections. Acute inflammation is mostly observed in the antrum and proceeds to chronic gastritis.

9.4.2 Chronic Gastritis

Histologic features of *H. pylori*-associated chronic gastritis are infiltration of various degrees of plasma cells, lymphocytes, and few eosinophils on lamina propria. These inflammatory cells are mostly distributed in the superficial and subepithelial layers of gastric mucosa. Generally, in normal gastric mucosa, two to three lymphocytes and/or plasma cells can be observed in lamina propria between foveolar glands, and approximately five intraepithelial lymphocytes are observed per 100 epithelial cells. If the infiltration of either lymphocyte or plasma cells is increased compared to the normal condition, the state can be diagnosed as chronic gastritis [8].

Neutrophilic infiltration is also observed; the infiltration is also mostly observed in the superficial and subepithelial layers of the gastric mucosa and causes intraepithelial neutrophils and pit microabscess by entering epithelial cells and lumen of gastric gland. If neutrophils are observed inside epithelial cells or gastric gland lumen, it is diagnosed as chronic active gastritis [8]. Neutrophilic infiltration is a common feature of *H. pylori* infection, and the degree of infiltration corresponds to the degree of *H. pylori* infection and mucosal damage. Moreover, neutrophilic infiltration is a crucial feature that indicates *H. pylori* infection.

Chronic gastritis is limited to the antral region during the initial stage, but it spreads to the body and fundus as the disease progresses (Fig. 9.2). In

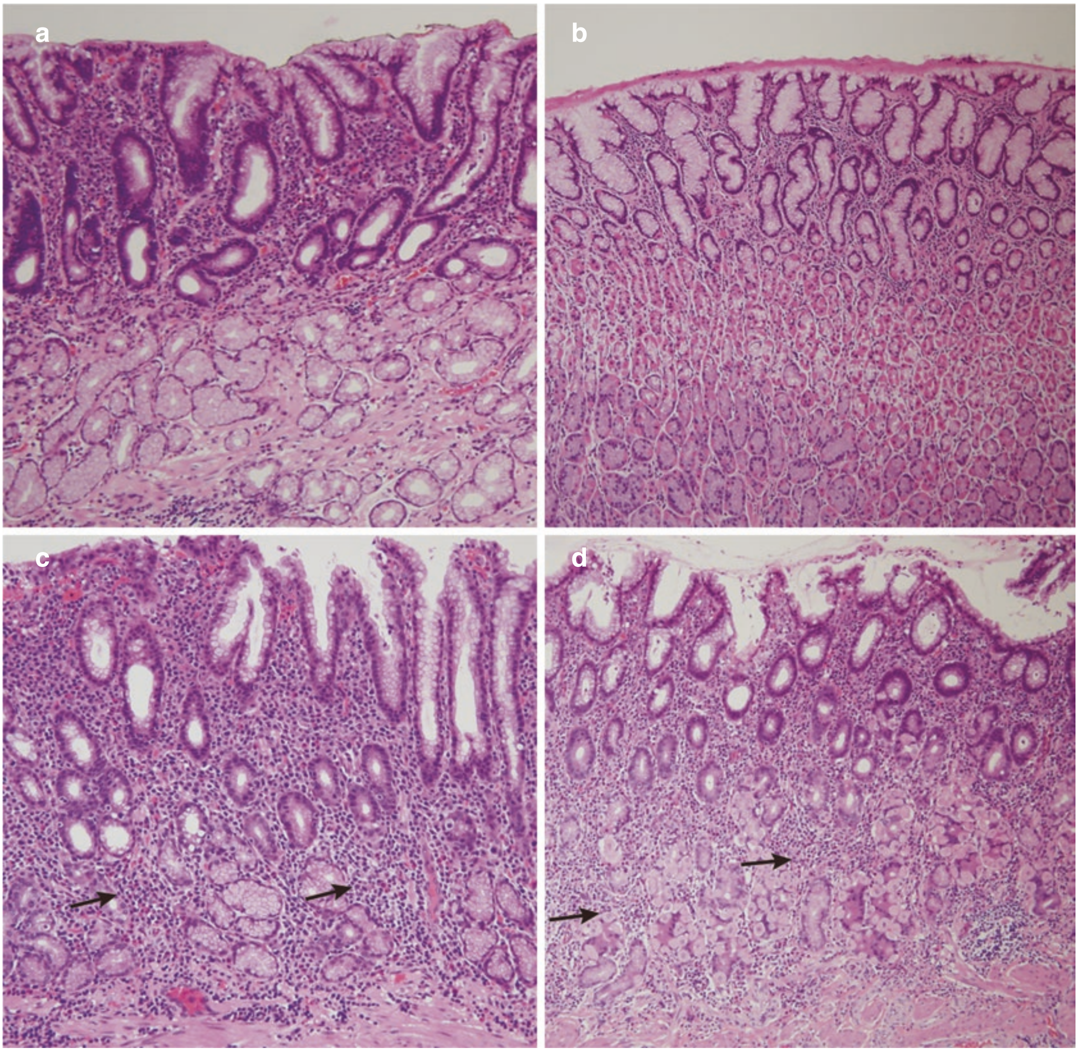

Fig. 9.2 Histopathologic features of chronic gastritis. (**a**) Superficial gastritis on gastric antral mucosa. (**b**) Superficial gastritis on gastric body mucosa. (**c**) Inflammatory cells infiltrate into the deep layer with the progression of gastritis on the antral region and destroy the glands (*arrow*). (**d**) Inflammatory cells infiltrate into the deep layer with the progression of gastritis on body mucosa and destroy the glands (*arrow*)

addition, inflammatory cell infiltration starts from the superficial layer of the gastric mucosa, and then, it enters the deeper area to induce parietal cell and chief cell loss. As a result, the body mucosa becomes similar to the antral mucosa or progresses to AG.

9.5 Sequelae of Chronic Gastritis

9.5.1 Atrophic Gastritis

AG is defined as the loss of appropriate glands in the gastric mucosa due to persistent chronic inflammation [27, 28]. Normally, the deep portion of gastric mucosal biopsy is composed of different cell types based on regions; the antral region is made of mucous glands, and the body is composed of parietal cells and chief cells that produce gastric juice and enzymes, which take part in gastric physiologic function. The loss of gastric deep glands is the diagnostic feature of AG. The superficial layer of the gastric mucosa is made of foveolar epithelium, and if IM is limited to foveolar epithelium, then it is not diagnosed as atrophy.

Atrophy can be classified into metaplastic type, which is due to IM or pseudopyloric metaplasia (Fig. 9.3a), and nonmetaplastic type, which is due to inflammation and/or stromal fibrosis. If a gastric biopsy specimen does not include the full thickness of the mucosal layer or is difficult to evaluate atrophy due to lymphoid follicle, it is diagnosed as “indefinite.” The degree of AG is categorized into mild, moderate, and severe based on the percentage of atrophic area/total area.

9.5.2 Intestinal Metaplasia

IM can be divided into two types [29–31]. First of all, the complete type shows similar histologic features as small intestine and is positive for MUC2 IHC, which is the mucin that is normally produced in the intestine. In comparison, the incomplete type shows similar histologic features as the large intestine and is positive to not only MUC2 but also MUC5AC and MUC6, which are normally produced in the stomach. In addition, high iron diamine-alcian blue (HID-AB) staining is a special stain that is used to distinguish those two types. After the HID-AB stain, sialomucin is stained in bright blue in the complete type, while sialomucin (bright blue) and sulfomucin (black) are both stained in the incomplete type. IM usually occurs with AG, and it is a precancerous lesion. There is a higher possibility of GC under the incomplete type of IM than the complete type.

9.5.3 Mucosa-Associated Lymphoid Tissue (MALT)

Well-formed lymphoid follicles are not observed in normal gastric mucosa, unlike small or large intestine. Sometimes, lymphocytes can accumulate on the upper portion of the muscularis mucosa. For *H. pylori*-positive patients, lymphoid follicles that are similar to intestinal MALT are observed (Fig. 9.3b). If lymphoid follicles are observed from gastric mucosal biopsy, *H. pylori* infection is highly suggested [8].

Antral nodular gastritis indicates that multiple small nodules are observed in gastric antrum by esophagogastroscopy but its histopathologic definition is still unclear. However, there are several reports that lymphoid follicle formation is observed as antral nodular gastritis [32, 33]. According to the recent research, small, circular, and elevated nodules are mainly observed on the gastric antrum via esophagogastroscopy, those nodules are flat on the body and fundus, and the nodules contain lymphoid follicles by histopathologic examination [34].

Moreover, MALT in the gastric mucosa is a cause of the marginal zone B-cell lymphoma of MALT. There was a report in the early 1990s that 92% of MALT lymphoma patients had *H. pylori* infection on their stomachs [35], and a recent research has reported that 70–95% of MALT lymphoma patients have *H. pylori* infection [36]. The histopathologic feature of low-grade MALT lymphoma shows that marginal zone B-cell-oriented tumor cells, which have irregular-shaped nuclei and pale cytoplasm (Fig. 9.4), infiltrate into the surrounding areas of lymphoid follicles.

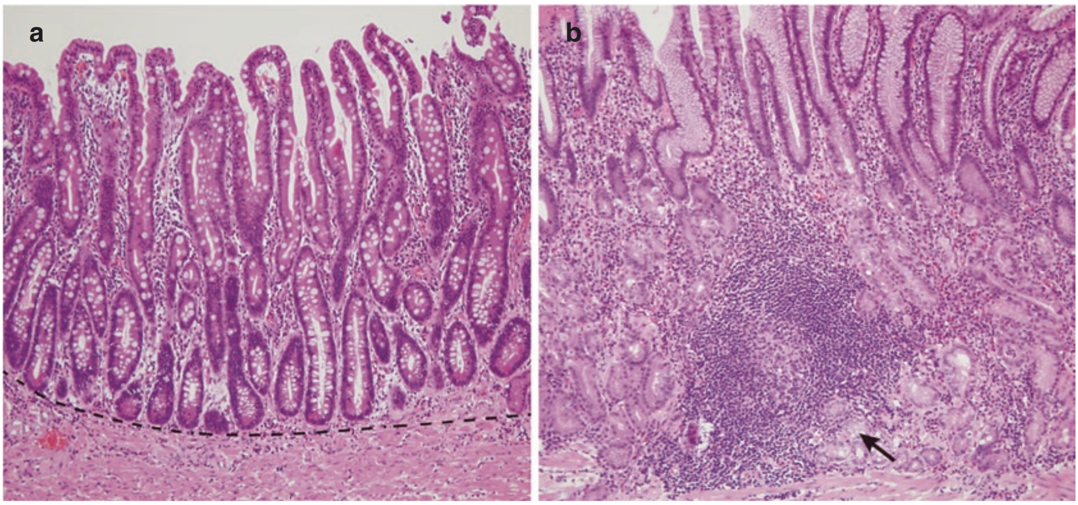

Fig. 9.3 Sequelae of chronic gastritis. (**a**) Atrophic gastritis is accompanied by intestinal metaplasia (*dotted line, upper border of muscularis mucosa*). (**b**) Mucosa-associated lymphoid tissue (MALT). A lymphoid follicle with a central germinal center is observed at the bottom of the mucosal layer (*arrow*)

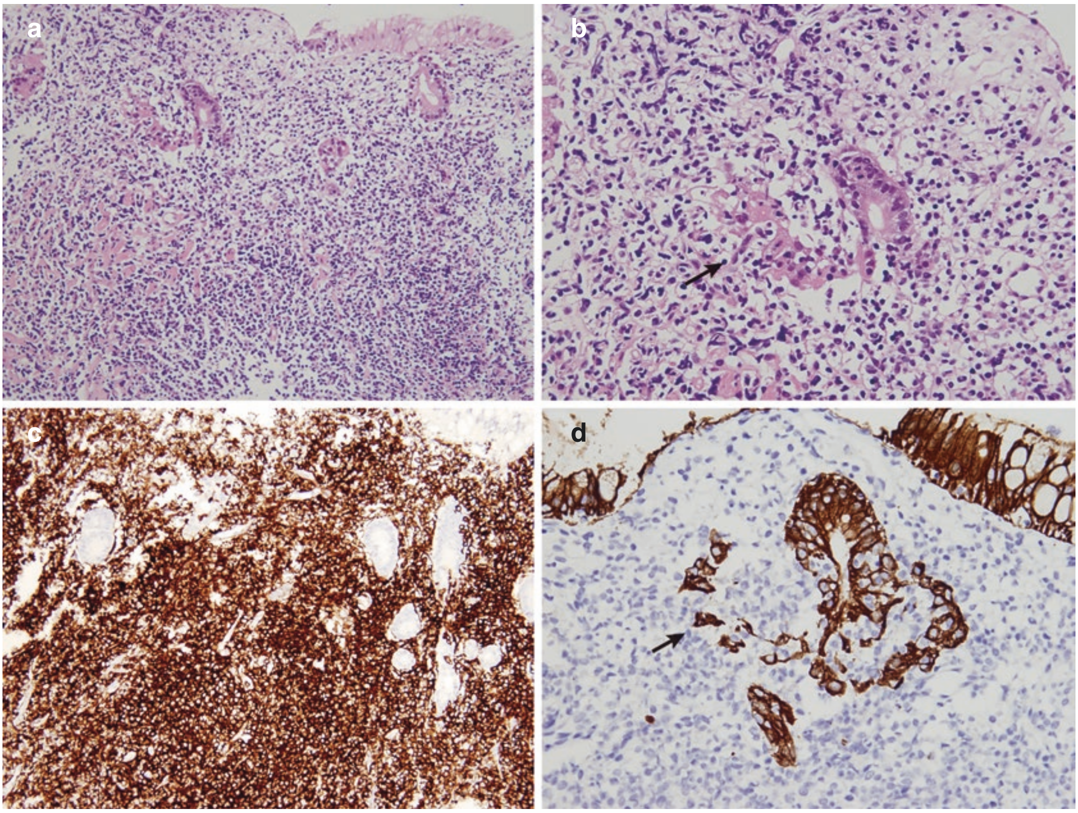

Fig. 9.4 Marginal zone of B-cell lymphoma of MALT (MALT lymphoma). (**a**) Diffuse infiltration of atypical lymphocyte is found in the mucosal layer of gastric biopsy specimen by H&E staining. (**b**) Lymphoepithelial lesion. Atypical lymphocytes are infiltrated into the gastric epithelium (*arrow*). (**c**) B-cell infiltration is confirmed by immunohistochemistry for CD20. (**d**) Destroyed gastric epithelium is easily observed by immunohistochemistry for cytokeratin (*arrow*)

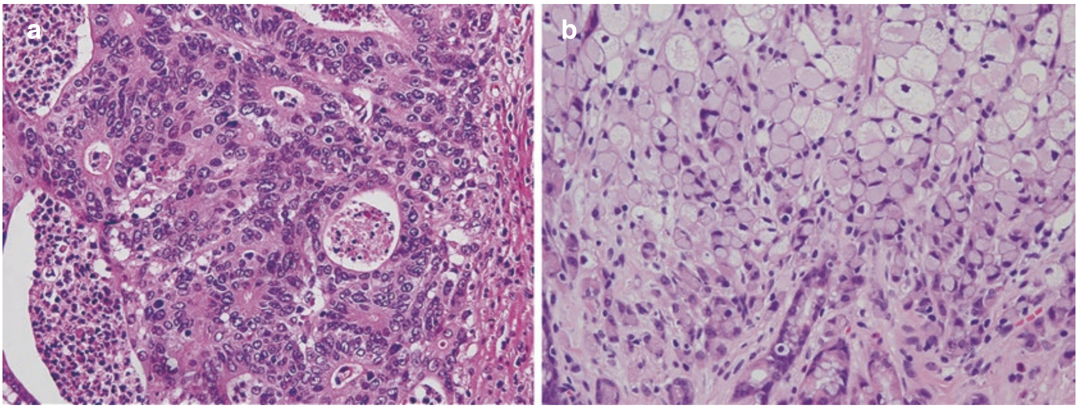

Fig. 9.5 Lauren classification in gastric cancer. (**a**) Intestinal type of gastric cancer. Tumor cells form a gland-like structure, which is similar to normal gastrointestinal glands. (**b**) Diffuse type of gastric cancer. Tumor cells scattered without forming gland structure

Remarkably, a lymphoepithelial lesion is observed, and this is a diagnostic finding of MALT lymphoma. Lymphoepithelial lesion is defined as infiltration of more than three neoplastic lymphocytes into surrounding gastric glands and destroys them. Ancillary IHC testing can verify the destruction of cytokeratin-positive glands and infiltration of CD20-positive neoplastic B cells. Intraepithelial lymphocytes can be also observed inside gastric glands in lymphocytic gastritis. In lymphocytic gastritis, T lymphocytes infiltrate into gastric glands, and those lymphocytes are scattered, not accumulated in more than three cells.

9.5.4 Gastric Cancer

The relationship between *H. pylori* infection and GC can be accepted by (1) a correlation between *H. pylori* infection and GC by epidemiologic studies, (2) sequential alterations of gastric mucosal lesion from *H. pylori* infection to cancer emergence with correa cascade, and (3) animal research model established [37]. In 1965, Lauren classified the GC into intestinal type and diffuse type [38]. Lauren classification is still used in clinical practice along with WHO classification because it is easy to classify and related to carcinogenesis and molecular genetic features (Fig. 9.5). The intestinal type occurs when tumor cells form gland structure that shows similar morphological features to normal gastrointestinal epithelium, whereas the diffuse type occurs when tumor cells are scattered and rarely form gland structure [39]. *H. pylori* infection and associated chronic gastritis cause AG and IM and then result in intestinal-type GC, but diffuse-type GC can also occur in correlation with *H. pylori* infection [37].

9.6 Pathologic Findings of Peptic Ulcer

Peptic ulcer is also an *H. pylori* infection-associated disease. Histologically, erosion indicates tissue damage that is limited to the mucosal layer, and ulcer indicates that the depth of tissue damage is below the submucosa layer. The peptic ulcer is composed of four layers based on histological observation: (1) fibrinoid ulcer at the surface layer, (2) mainly neutrophils but nonselective inflammatory cell infiltration layer, (3) monocytic infiltration and capillary proliferation, and (4) fibrosis in the deepest layer.

9.7 Conclusion

H. pylori can be detected by several diagnostic methods, and histopathologic examination is one of those. H&E stain is a commonly used method in histopathologic diagnosis that does not require additional staining procedures and can obtain other histological findings, but it has low sensitivity and specificity in terms of *H. pylori* detection. The intraepithelial neutrophilic infiltration and pit abscess are distinctive features of *H. pylori*-associated gastritis, and a special stain or IHC is helpful if there is significant inflammation but no *H. pylori* detection. Giemsa stain is a quick and easy method with high sensitivity, and silver stain and IHC have high sensitivity and specificity but labor intensive and time-consuming. With recent advances in diagnostic techniques, several molecular tests have been developed to detect *H. pylori* and clarithromycin resistance, especially PCR-based methods. Each pathologic laboratory is recommended to choose the proper staining method based on its facility and human resources.

Persistent chronic gastritis due to *H. pylori* infection results in AG, IM, and MALT as sequelae. Most of them are precancerous lesions that should be reported in histopathologic diagnosis. In addition, *H. pylori* infection is related to MALT lymphoma or GC. Histopathologic diagnosis of *H. pylori* should include the degree of gastritis, sequela, and malignant tumor detection; therefore, histological diagnosis is necessary for gastritis patients.

References

1. McColl KE. Clinical practice. *Helicobacter pylori* infection. N Engl J Med. 2010;362:1597–604.
2. International Agency for Research on Cancer, World Health Organization. Infection with *Helicobacter pylori.* In: Schistosomes, liver flukes and *Helicobacter pylori.* Lyon: IARC Monogr Eval Carcinog Risks Hum; 1994. p. 177–240.
3. Basset C, Holton J, Ricci C, Gatta L, Tampieri A, Perna F, et al. Review article: diagnosis and treatment of Helicobacter: a 2002 updated review. Aliment Pharmacol Ther. 2003;17 (Suppl 2):89–97.
4. Rautelin H, Lehours P, Mégraud F. Diagnosis of *Helicobacter pylori* infection. Helicobacter. 2003;8 (Suppl 1):13–20.
5. Laine L, Lewin DN, Naritoku W, Cohen H. Prospective comparison of H&E, Giemsa, and Genta stains for the diagnosis of *Helicobacter pylori*. Gastrointest Endosc. 1997;45:463–7.
6. Fallone CA, Loo VG, Lough J, Barkun AN. Hematoxylin and eosin staining of gastric tissue for the detection of *Helicobacter pylori*. Helicobacter. 1997;2:32–5.
7. El-Zimaity HM, Segura AM, Genta RM, Graham DY. Histologic assessment of *Helicobacter pylori* status after therapy: comparison of Giemsa, Diff-Quik, and Genta stains. Mod Pathol. 1998;11:288–91.
8. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney system. International workshop on the histopathology of gastritis, Houston 1994. Am J Surg Pathol. 1996;20:1161–81.
9. el-Zimaity HM, Wu J, Akamatsu T, Graham DY. A reliable method for the simultaneous identification of *H. pylori* and gastric metaplasia in the duodenum. J Clin Pathol. 1999;52:914–6.
10. Genta RM, Robason GO, Graham DY. Simultaneous visualization of Helicobacter pylori and gastric morphology: a new stain. Hum Pathol. 1994;25:221–6.
11. Doglioni C, Turrin M, Macri E, Chiarelli C, Germanà B, Barbareschi M. HpSS: a new silver staining method for *Helicobacter pylori*. J Clin Pathol. 1997;50:461–4.
12. Mendoza ML, Martín-Rabadán P, Carrión I, Morillas JD, López-Alonso G, Diaz-Rubio M. *Helicobacter pylori* infection. Rapid diagnosis with brush cytology. Acta Cytol. 1993;37:181–5.
13. Ranjbar R, Ebrahimi A, Sahebkar A. *Helicobacter pylori* infection: conventional and molecular strategies for bacterial diagnosis and antibiotic resistance testing. Curr Pharm Biotechnol. 2023;24:647–64.
14. Seo TH, Lee SY, Uchida T, Fujioka T, Jin CJ, Hwang TS, et al. The origin of non-*H. pylori*-related positive Giemsa staining in human gastric biopsy specimens: a prospective study. Dig Liver Dis. 2011;43:23–7.
15. Jonkers D, Stobberingh E, de Bruine A, Arends JW, Stockbrügger R. Evaluation of immunohistochemistry for the detection of *Helicobacter pylori* in gastric mucous membrane biopsies. J Infect. 1997;35:149–54.
16. Andersen LP, Rasmussen L. *Helicobacter pylori*-coccoid forms and biofilm formation. FEMS Immunol Med Microbiol. 2009;56:112–5.
17. Riddell R, Jain D. Lewin, Weinstein and Riddell's gastrointestinal pathology and its clinical implications. 2nd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2014.
18. Sousa C, Ferreira R, Santos SB, Azevedo NF, Melo LDR. Advances on diagnosis of *Helicobacter pylori* infections. Crit Rev Microbiol. 2023;49:671–92.
19. Versalovic J, Shortridge D, Kibler K, Griffy MV, Beyer J, Flamm RK, et al. Mutations in 23S rRNA are associated with clarithromycin resistance in

Helicobacter pylori. Antimicrob Agents Chemother. 1996;40:477–80.

20. Woo HY, Park DI, Park H, Kim MK, Kim DH, Kim IS, et al. Dual-priming oligonucleotide-based multiplex PCR for the detection of *Helicobacter pylori* and determination of clarithromycin resistance with gastric biopsy specimens. Helicobacter. 2009;14:22–8.
21. Lehours P, Siffré E, Mégraud F. DPO multiplex PCR as an alternative to culture and susceptibility testing to detect *Helicobacter pylori* and its resistance to clarithromycin. BMC Gastroenterol. 2011;11:112.
22. Nahm JH, Kim WK, Kwon Y, Kim H. Detection of *Helicobacter pylori* with clarithromycin resistance-associated mutations using peptide nucleic acid probe-based melting point analysis. Helicobacter. 2019;24:e12634.
23. Noh JH, Ahn JY, Choi J, Park YS, Na HK, Lee JH, et al. Real-time polymerase chain reaction for the detection of *Helicobacter pylori* and clarithromycin resistance. Gut Liver. 2023;17:375–81.
24. Ramírez-Lázaro MJ, Lario S, Quílez ME, Montserrat A, Bella MR, Junquera F, et al. Droplet digital PCR detects low-density infection in a significant proportion of *Helicobacter pylori*-negative gastric biopsies of dyspeptic patients. Clin Transl Gastroenterol. 2020;11:e00184.
25. Sun L, Talarico S, Yao L, He L, Self S, You Y, et al. Droplet digital PCR-based detection of clarithromycin resistance in *Helicobacter pylori* isolates reveals frequent heteroresistance. J Clin Microbiol. 2018;56:e00019–8.
26. Kim JJE, Kocsmár I, Buzás GM, Szirtes I, Rusz O, Diczházi C, et al. Efficacy of clarithromycin depends on the bacterial density in clarithromycin-heteroresistant *Helicobacter pylori* infections: an *in situ* detected susceptibility and quantitative morphometry-based retrospective study. Pathol Oncol Res. 2021;27:1609863.
27. Rugge M, Correa P, Dixon MF, Fiocca R, Hattori T, Lechago J, et al. Gastric mucous membrane atrophy: interobserver consistency using new criteria for classification and grading. Aliment Pharmacol Ther. 2002;16:1249–59.
28. Rugge M, Correa P, Di Mario F, El-Omar E, Fiocca R, Geboes K, et al. OLGA staging for gastritis: a tutorial. Dig Liver Dis. 2008;40:650–8.
29. Filipe MI, Muñoz N, Matko I, Kato I, Pompe-Kirn V, Jutersek A, et al. Intestinal metaplasia types and the risk of gastric cancer: a cohort study in Slovenia. Int J Cancer. 1994;57:324–9.
30. Rokkas T, Filipe MI, Sladen GE. Detection of an increased incidence of early gastric cancer in patients with intestinal metaplasia type III who are closely followed up. Gut. 1991;32:1110–3.
31. Liu Q, Teh M, Ito K, Shah N, Ito Y, Yeoh KG. CDX2 expression is progressively decreased inhuman gastric intestinal metaplasia, dysplasia and cancer. Mod Pathol. 2007;20:1286–97.
32. Miyamoto M, Haruma K, Yoshihara M, Hiyama T, Sumioka M, Nishisaka T, et al. Nodular gastritis in adults is caused by *Helicobacter pylori* infection. Dig Dis Sci. 2003;48:968–75.
33. Hassall E, Dimmick JE. Unique features of *Helicobacter pylori* disease in children. Dig Dis Sci. 1991;36:417–23.
34. Hayashi S, Imamura J, Kimura K, Saeki S, Hishima T. Endoscopic features of lymphoid follicles in *Helicobacter pylori*-associated chronic gastritis. Dig Endosc. 2015;27:53–60.
35. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. Lancet. 1991;338:1175–6.
36. Sumida T, Kitadai Y, Hiyama T, Shinagawa K, Tanaka M, Kodama M, et al. Antibodies to *Helicobacter pylori* and CagA protein are associated with the response to antibacterial therapy in patients with *H. pylori*-positive API2-MALT1-negative gastric MALT lymphoma. Cancer Sci. 2009;100:1075–81.
37. Tan D, Lauwers GY. Advances in surgical pathology: gastric cancer. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2011. 1 Har/Psc ed.
38. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand. 1965;64:31–49.
39. Bosman FT, Carneiro F, Hruban RH, Theise ND. The International Agency for Research on Cancer. WHO classification of tumours of the digestive system. 4th ed. Lyon: IARC Press; 2010.

Culture

10

Nayoung Kim and Jaeyeon Kim

Abstract

The culture of *Helicobacter pylori* (*H. pylori*) is an accurate method for the diagnosis of *H. pylori* and a necessary step for antimicrobial susceptibility testing. In the optimal culture condition, 35–37 °C in the microaerophilic condition (85% N_2, 10% CO_2, 5% O_2), colonies usually appear in 3–4 days. However, in the case of negative culture, 7–10 days of incubation is recommended to make sure that the result is negative. As it is very expensive and fastidious, culture study is limited to research. There are several methods for antimicrobial susceptibility testing after culture of *H. pylori* such as agar dilution method, disk diffusion method, broth dilution method, and E-test. Among these methods, the agar dilution method is regarded as a standard method internationally. This antimicrobial susceptibility testing is comprehensive for multiple antibiotics. However, as the agar dilution method is time-consuming and expensive, antimicrobial susceptibility testing has been recommended after the second failure of *H. pylori* eradication. In spite of these difficulties of agar dilution method, due to gradual increase of antimicrobial resistance, recent guidelines suggest that culture with antimicrobial susceptibility testing could be essential for the initial treatment of peptic ulcer diseases, gastric extranodal marginal zone B-cell lymphomas of mucosa-associated lymphoid tissue (MALT), and gastric cancer in the future.

Keywords

Helicobacter pylori · Culture · Diagnosis
Antimicrobial susceptibility testing

10.1 Introduction

Bacterial culture is a comprehensive method for antimicrobial susceptibility testing and typing of strains [1]. In the optimal condition, the specificity is 100% and the sensitivity is more than 90%. However, *Helicobacter pylori* (*H. pylori*) is a fastidious bacterium whose isolation rate varies according to the collection and transport of specimens and selection of culture medium; the rather low sensitivity has been reported in 50–70% according to the researchers [2–5]. Especially in patients with gastrointestinal bleeding, it has been reported in 40% [6]. The sensitivity is 95.8%, and specificity is reported 96.4% in children [7]. The most difficult thing is that the

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culture yield of *H. pylori which* is rather low because *H. pylori* slowly grows under anaerobic and humid conditions. That is, because of the delicate and fastidious nature of *H. pylori*, the cultivation in vitro requires a particular transport medium, growth medium, and incubation environment [8]. Although culture is a time-consuming, expensive, and laborious test for *H. pylori* diagnosis, the antibiotic sensitivity test results of *H. pylori* provided by culture are precious information in clinical practice [8]. As recommended by the Maastricht VI Consensus Report, *H. pylori* culture and antibiotic susceptibility testing should be performed if primary resistance to clarithromycin is higher than 20% in a given geographical area or after failure of second-line treatment [9]. Furthermore, culture also allows isolation of *H. pylori* for further analysis of phenotypic and genotypic characterization to have a better understanding of the pathogens and, consequently, offer therapy evaluation [8]. With the increasing prevalence of antibiotic resistance, culturing is still a reliable method for managing *H. pylori* treatment failure as well as surveying antibiotic resistance in population-based studies before other molecular tests are more widely available [10].

10.2 Culture Method

Conditions such as poor quality of specimens, delayed transport, exposure to the aerobic environment, or inexperienced microbiologists have adverse influences on the performance of culture and reduce diagnostic accuracy [11].

10.2.1 Specimen Collection

The proper specimen for culturing *H. pylori* is biopsy specimens obtained during endoscopy. Host factors like high activity of gastritis, low bacterial load, bleeding, alcohol drinking, and use of H_2-receptor antagonists, proton pump inhibitors (PPI), and antibiotics have adverse effects on the culture-positive rate [8]. These medications, except for antibiotics that should be avoided at least 4 weeks, are also suggested to be avoided 2 weeks before culture. Since strong acid blockers such as PPI or potassium-competitive acid blockers (P-CAB) change the distribution of mucosal bacteria [12], subjects who are scheduled to undergo endoscopy should quit PPIs or P-CAB at least for 2 weeks or more.

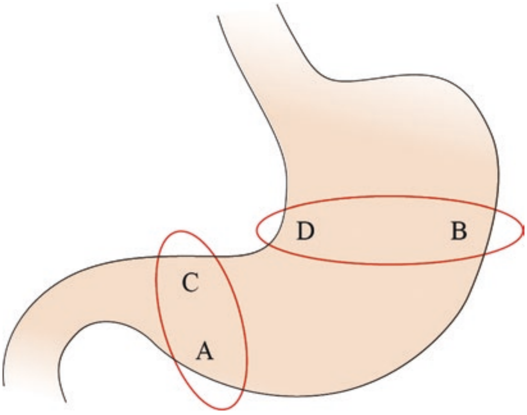

Fig. 10.1 For *Helicobacter pylori* isolation, gastric biopsy specimens are obtained from the mid antrum (two pieces, one from the lesser curvature [**C**] and one from the greater curvature [**A**]) and mid corpus (two pieces, one from the lesser curvature [**D**] and one from the greater curvature [**B**]) of the stomach during endoscopic examinations

There is controversy over the number of tissue samples required for the diagnosis of *H. pylori*. A single biopsy at the antrum (2 cm from the pylorus) is sensitive, but it is not sufficient for a reliable diagnosis [13]. As *H. pylori* may have patch distribution, especially in the presence of intestinal metaplasia, the larger number of samples taken increases the sensitivity. Therefore, it is recommended to take two specimens each on antrum and corpus [13, 14] (Fig. 10.1). Between endoscopies, endoscopes (including biopsy channels and forceps) are cleaned thoroughly with detergent and disinfected for 30 min.

10.2.2 Transport of Biopsy Specimens

H. pylori is fragile in room temperature and air. It is obligatory to avoid air exposure of the specimen as soon as taking a biopsy and to place them in saline or transport media, such as Stuart's transport medium if transport time is more than 4 h [13, 15]. The biopsies are soaked in brucella broth (Difco Laboratories) or Stuart's transport medium to avoid getting dry during delivery to the culture bench. If these transport conditions cannot be available, it is better to freeze the specimens at −70 °C. When stored at 4 °C in media containing 20% glycerol, it is known that 81% of *H. pylori* had been recovered [16].

A recent study conducted in 26 hospitals to analyze the influence of transport time as well as temperature on culture rate showed that the positive culture rate decreased to 26.3% in 48 h transport group as compared to 32.8% in 24 h transport group ($p < 0.001$) [17]. This study also found that the average temperature increased from 4.7 to 29.1 °C during transportation, resulting in the decrease of positive culture rate from 36.7% to 24.1% [17]. Recently developed GESA transport medium [8] is a semisolid medium that can store gastric biopsy specimens at 4 °C for up to 10 days and provide a quantifiable recovery rate of *H. pylori* (90.7%) [18]. A new biphasic test, which combined the selective enrichment broth and biochemical test using urea agar in a single vessel, was also developed for culturing *H. pylori* in gastric biopsies [8]. In this small study, the biphasic test was conducted in 55 biopsy specimens and showed a 100% positive predictive valve after 48 h incubation [19]. Moreover, this method had a lower false positive rate and required a lower bacterial load, approximately 10^5 colony-forming units (CFU)/mL, as compared with rapid urease tests such as CLOtest. At the same time, this test could be used under an aerobic condition and allowed culturing as well as antibiotic susceptibility testing [19].

10.2.3 Incubation

H. pylori is not distributed evenly in most cases; more colonies appear in the ground specimens. In addition, direct plating to a solid medium is used as *H. pylori* is difficult to grow in the broth culture. At first, light pipetting of media in which biopsy material is contained properly is performed. With a flattened microtip, grind biopsy specimens and then streak (Fig. 10.2a).

The commonly used media include Pylori agar, Skirrow agar, Columbia blood agar, Brucella agar, Brain heart infusion, or Trypticase soy agar [8]. For example, the media include an agar base, growth supplements, and selective supplements. Blood or serum components are examples of growth supplements, which promote the growth of *H. pylori*, and the proportion is 5%, 7%, or 10%. Other growth supplements include yolk, charcoal, starch, bovine serum albumin, catalase, and so on [13]. Selective supplements are crucial due to the presence of contaminating bacteria. It consists of antibiotics and antifungal: antibiotics that inhibit the growth of Gram-positive bacteria such as vancomycin or teicoplanin, antibiotics targeting Gram-negative bacteria such as nalidixic acid colistin or trimethoprim, and antifungal such as nystatin or amphotericin B. To increase the sensitivity of the test, a selective medium or the blood containing nonselective medium is used [20]. Selective medium, such as Pylori agar and Skirrow agar, and nonselective medium, such as blood agar and Columbia agar, are frequently used [21]. Usually incubating the bacteria cultures is performed in microaerobic conditions of 35–37 °C (85% N_2, 10% CO_2, 5% O_2), and the colonies appear within 3–4 days in optimal conditions. However, 7–10 days of incubation is recommended to make sure that the result is negative [22]. Although *H. pylori* has been considered as a microaerophile, a recent study showed that the growth of *H. pylori* has been promoted by atmospheric oxygen levels with the presence of 10% CO_2, bringing a novel concept that *H. pylori* may be a capnophilic aerobe [23].

10.2.4 Identification

After 3–4 days in the culture medium, transparent and smooth colonies of small round *H. pylori* can be identified [13] (Fig. 10.2b). If the bacteria are separated from the gastric biopsy samples, it is sufficient to identify the phenotype but not

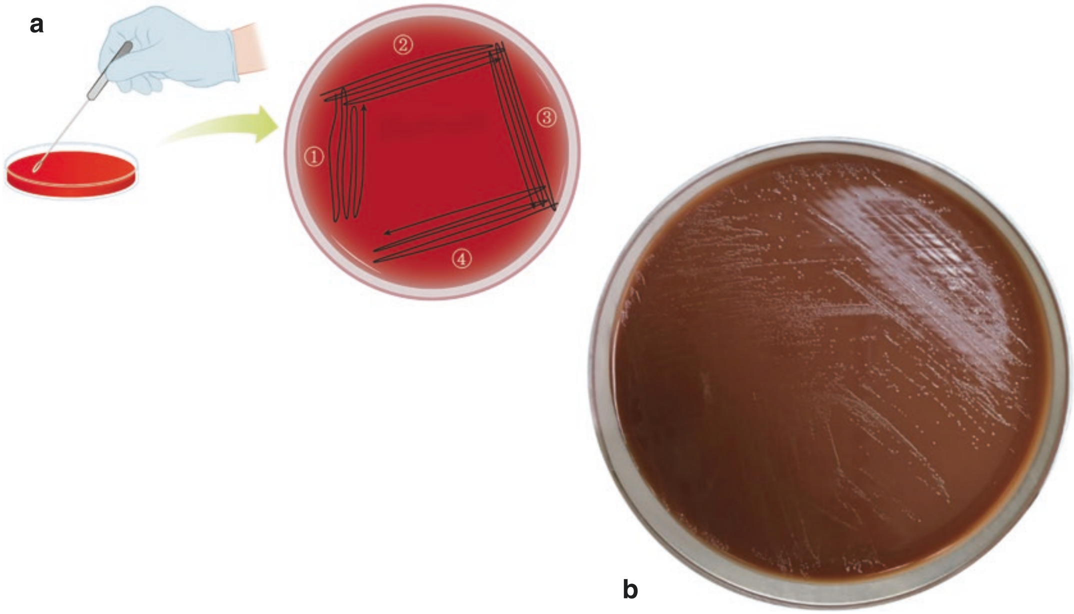

Fig. 10.2 *H. pylori* culture. (**a**) (1) Add 200 μL of *Brucella* broth to petri dishes. (2) Put two pieces of biopsy specimens from the gastric antrum and corpus on each petri dish. (3) After heating the end of the microtip, put it flattened on petri dishes. (4) With a flattened microtip, grind biopsy specimens and then streak from ① to ④ directions over the Pylori agar. (5) Incubate at 37 °C, 10% CO_2, 5% O_2 condition for 3–5 days. (**b**) After 3–4 days in the culture medium, transparent and smooth colonies of small round *H. pylori* can be identified on the Pylori agar

from a stool or salivary specimen. To differentiate other bacteria with similar characteristics, molecular biological method is used [24]. Diagnosis of *H. pylori* from culture medium is based on morphological characteristics as well as positive urease, catalase, and oxidase reactions, which means the microbiological laboratories should be equipped and trained to isolate this bacterium [8].

10.3 Antimicrobial Susceptibility Testing

After isolation of *H. pylori* (Fig. 10.3), to assess antimicrobial susceptibility, agar dilution method, disk diffusion method, broth dilution method, and E-test are performed [25–27]. Clinical Laboratory Standard Institute (CLSI) has recommended an agar dilution test as the standard for testing the antimicrobial susceptibility of *H. pylori* [28].

10.3.1 Agar Dilution Method

Add 5% of blood or serum of sheep or horses based on Mueller–Hinton agar (MHA) with prepared twofold dilutions of antibiotics. Then, inoculate the isolated *H. pylori* on agar at 35–37 °C with 72 h of incubation [28, 29] (Fig. 10.4).

About 5% of blood containing MHA is prepared with twofold dilutions of antibiotics depending on each antibiotic (Fig. 10.5). The target antibiotic drug is related with the clinically used eradication treatments such as PPI-based triple therapy, bismuth-based quadruple therapy, or rifabutin-based triple therapy. The minimum concentration that *H. pylori* does not grow is determined as minimal inhibitory concentration (MIC).

Two-fold dilution of each antibiotic could be different depending on the antibiotic resistance (Fig. 10.6). Usually the cutoff value of antibiotic resistance is based on the guideline of CLSI. This

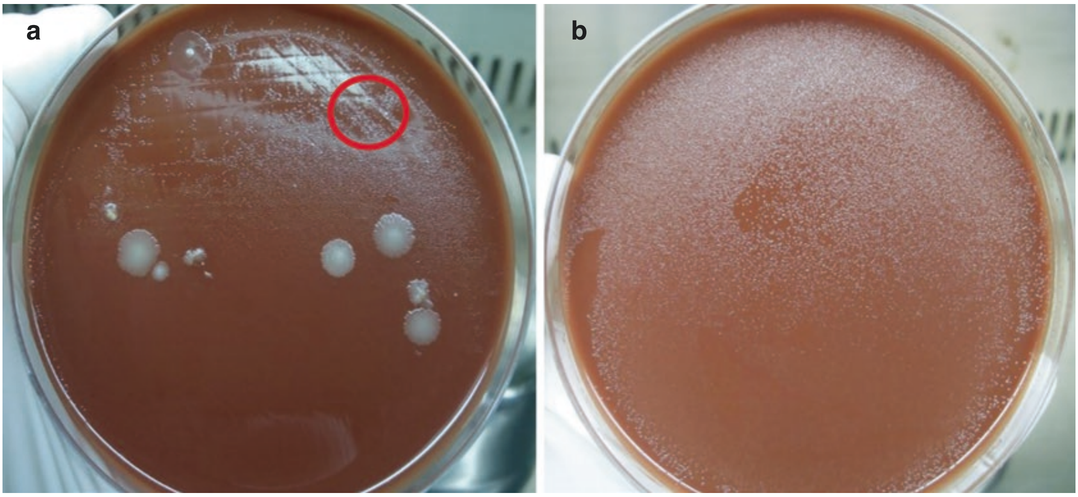

Fig. 10.3 Isolation of *H. pylori*. (**a**) Following the procedure in Fig. 10.2. The culture is considered to be positive for *H. pylori* by observing small, transparent, tiny colonies (*red oval*). (**b**) Repeat this step of inoculating and isolation three times on the selective media including appropriate antibiotics. Mass culture of the fourth isolated *H. pylori* after checking urease positive and Gram negative. Now it is ready to perform a minimal inhibitory concentration (MIC) test

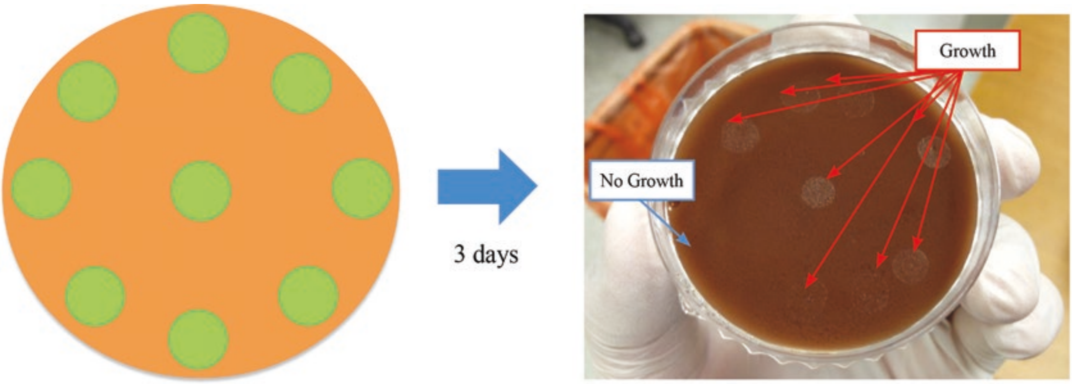

Fig. 10.4 Key concept of agar dilution method for antibiotic resistance of *H. pylori*

method is useful in research by observing antibiotic resistance of many strains at the same time in the laboratory. However, it is not frequently used in the clinic.

10.3.2 Disk Diffusion Method

The disk diffusion method is applicable only to bacteria which can form a colony in 1 day; slow-growing bacteria should check the broth dilution method. This method has not been validated for the other antibiotics except macrolide, but a good correlation is usually found with the other methods [13, 29].

10.3.3 Broth Dilution Method

The antibiotics diluted in twofold serial manner are added in the liquid culture medium by inoculating the microorganism. The lowest antibiotic concentration in a test tube that bacteria do not grow is an MIC. Strains should be used as a

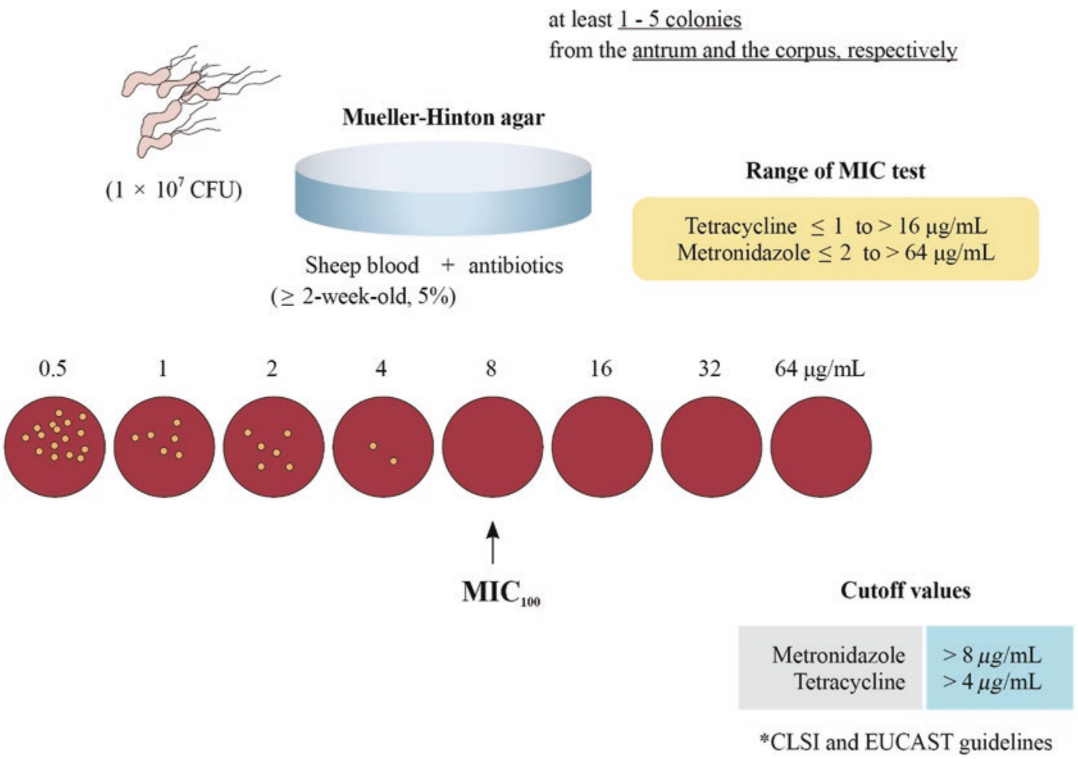
at least 1 - 5 colonies
from the antrum and the corpus, respectively
(1 × 10⁷ CFU)
Mueller-Hinton agar
Sheep blood + antibiotics
(≥ 2-week-old, 5%)
Range of MIC test
Tetracycline ≤ 1 to > 16 μg/mL
Metronidazole ≤ 2 to > 64 μg/mL
0.5
1
2
4
8
16
32
64 μg/mL
MIC100
Cutoff values
Metronidazole > 8 μg/mL
Tetracycline > 4 μg/mL
*CLSI and EUCAST guidelines

Fig. 10.5 Testing antimicrobial susceptibility of *H. pylori* with the agar dilution method. Adding 5% of blood on Mueller–Hinton agar with prepared twofold dilutions of antibiotics. Then, inoculate the isolated *H. pylori* on agar at 35–37 °C with 72 h of incubation. The concentration range is made up of 1–64 μg/mL or 0.125–2 μg/mL for each antibiotic, and the minimum concentration that *H. pylori* does not grow is determined as minimal inhibitory concentration (MIC). *CLSI* Clinical Laboratory Standard Institute

known MIC for quality control. As *H. pylori* are difficult to grow in the liquid medium, *Brucella* is a helpful supplementation. It shows relative good correlation with the E-test except metronidazole [27].

10.3.4 E-Test

E-test strip is a thin inert, width 5 mm and length 50 mm, of plastic that do not have holes. The antibiotic concentration is recorded on one side of the strip, and the concentration range is made up of 1–32 μg/mL or 0.125–2 μg/mL for each antibiotic. Put the three to five colonies of bacteria to be tested in a liquid medium, and coat the bacteria on MHA according to McFarland standard 0.5. Then put the strip on MHA. When cultured in constant temperature of 35 °C in the absence of CO_2, there will be symmetrical inhibition zone elliptical around the strip. The edge of strips of oval intersection is the MIC values. E-test of antibiotic sensitivity testing of *H. pylori* and the agar diffusion method were reported to have relatively good correlation except metronidazole [27].

Antibiotics	Unit : μg/mL								
Amoxicillin				2	1	0.5	0.25	0.125	
Tetracycline				16	8	4	2	1	
Metronidazole			64	32	16	8	4	2	
Levofloxacin		16	8	4	2	1	0.5	0.25	0.125
Moxifloxacin		16	8	4	2	1	0.5	0.25	0.125
Clarithromycin	32	16	8	4	2	1	0.5	0.25	
Rifabutin				1	0.5	0.25	0.125	0.06	

Fig. 10.6 Two-fold dilution of each antibiotic in the agar dilution method. The range of tests for each antibiotic could be different depending on the antibiotic resistance state of each country

10.4 Conclusions

Although culture is a time-consuming, expensive and laborious test for *H. pylori* diagnosis, the antibiotic sensitivity test of *H. pylori* provided after successful culture is a particular advantage in clinical practice. This culture of *H. pylori* is influenced by experiences of microbiologists, quality of specimen, and transportation media. Thus clinically its role has been limited to check antimicrobial susceptibility in patients who have failed more than twice in eradication. However, culture also allows isolation of *H. pylori* for further analysis of phenotypic and genotypic characterization to have better understanding of the pathogens and offers therapy evaluation opportunity [8]. With the increasing prevalence of antibiotic resistance, culturing is still a reliable method for managing *H. pylori* treatment failure as well as surveying antibiotic resistance in population-based studies before other molecular tests are more widely available.

References

1. Hirschl AM, Makristathis A. Methods to detect *Helicobacter pylori*: from culture to molecular biology. Helicobacter. 2007;12:6–11.
2. Glupczynski Y. Microbiological and serological diagnostic tests for *Helicobacter pylori*: an overview. Br Med Bull. 1998;54:175–86.
3. van Zwet AA, Thijs JC, Roosendaal R, Kuipers EJ, Peña S, de Graaff J. Practical diagnosis of *Helicobacter pylori* infection. Eur J Gastroenterol Hepatol. 1996;8:501–7.
4. Grove DI, Koutsouridis G, Cummins AG. Comparison of culture, histopathology and urease testing for the diagnosis of *Helicobacter pylori* gastritis and susceptibility to amoxicillin, clarithromycin, metronidazole and tetracycline. Pathology. 1998;30:183–7.
5. Loffeld RJ, Stobberingh E, Flendrig JA, Arends JW. *Helicobacter pylori* in gastric biopsy specimens. Comparison of culture, modified Giemsa stain, and immunohistochemistry. A retrospective study. J Pathol. 1991;165:69–73.
6. Choi YJ, Kim N, Lim J, Jo SY, Shin CM, Lee HS, et al. Accuracy of diagnostic tests for *Helicobacter pylori* in patients with peptic ulcer bleeding. Helicobacter. 2012;17:77–85.

7. Mendoza-Ibarra SI, Perez-Perez GI, Bosques-Padilla FJ, Urquidi-Rivera M, Rodríguez-Esquivel Z, Garza-González E. Utility of diagnostic tests for detection of *Helicobacter pylori* in children in northeastern Mexico. Pediatr Int. 2007;49:869–74.
8. Wang YK, Kuo FC, Liu CJ, Wu MC, Shih HY, Wang SS, et al. Diagnosis of *Helicobacter pylori* infection: current options and developments. World J Gastroenterol. 2015;21:11221–35.
9. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection—the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
10. Godbole G, Mégraud F, Bessède E. Review: diagnosis of *Helicobacter pylori* infection. Helicobacter. 2020;25 (Suppl 1):e12735.
11. Ndip RN, MacKay WG, Farthing MJ, Weaver LT. Culturing *Helicobacter pylori* from clinical specimens: review of microbiologic methods. J Pediatr Gastroenterol Nutr. 2003;36:616–22.
12. Mégraud F, Boyanova L, Lamouliatte H. Activity of lansoprazole against *Helicobacter pylori*. Lancet. 1991;337:1486.
13. Mégraud F, Lehours P. *Helicobacter pylori* detection and antimicrobial susceptibility testing. Clin Microbiol Rev. 2007;20:280–322.
14. Lee JW, Kim N, Choi SI, Jang JY, Song CH, Nam RH, et al. Prevalence and trends of multiple antimicrobial resistance of *Helicobacter pylori* in one tertiary hospital for 20 years in Korea. Helicobacter. 2023;28:e12939.
15. Meunier O, Walter P, Chamouard P, Piemont Y, Monteil H. Isolation of *Helicobacter pylori*: necessity of control of transport conditions. Pathol Biol. 1997;45:82–5.
16. Han SW, Flamm R, Hachem CY, Kim HY, Clarridge JE, Evans DG, et al. Transport and storage of *Helicobacter pylori* from gastric mucosal biopsies and clinical isolates. Eur J Clin Microbiol Infect Dis. 1995;14:349–52.
17. Gong YN, Li YM, Yang NM, Li HZ, Guo F, Lin L, et al. Centralized isolation of *Helicobacter pylori* from multiple centers and transport condition influences. World J Gastroenterol. 2015;21:944–52.
18. Cellini L, Di Campli E, Di Bartolomeo S, Bessa LJ, Baffoni M, Di Giulio M. New transport medium for cultural recovery of *Helicobacter pylori*. J Clin Microbiol. 2014;52:4325–9.
19. Wisessombat S, Meethai C, Hamgo S. A new biphasic test for the detection of *Helicobacter pylori* in gastric biopsies. J Microbiol Methods. 2014;96:19–24.
20. Piccolomini RP, Bonaventura GD, Festi D, Catamo G, Laterza F, Neri M. Optimal combination of media for primary isolation of *Helicobacter pylori* from gastric biopsy specimens. J Clin Microbiol. 1997;35:1541–4.
21. Garza-González E, Perez-Perez GI, Maldonado-Garza HJ, Bosques-Padilla FJ. A review of *Helicobacter pylori* diagnosis, treatment, and methods to detect eradication. World J Gastroenterol. 2014;20:1438–49.
22. van der Hulst RW, Verheul SB, Weel JF, Gerrits Y, ten Kate FJ, Dankert J, et al. Effect of specimen collection techniques, transport media and incubation of cultures on the detection rate of *Helicobacter pylori*. Eur J Clin Microbiol Infect Dis. 1996;15:211–5.
23. Park SA, Ko A, Lee NG. Stimulation of growth of the human gastric pathogen *Helicobacter pylori* by atmospheric level of oxygen under high carbon dioxide tension. BMC Microbiol. 2011;11:96.
24. Lim TS, Ho B. A computer program in quick basic for the selection of tests for the identification of *Helicobacter pylori*. Comput Biol Med. 1993;23:21–7.
25. Hardy DJ, Hanson CW, Hensey DM, Beyer JM, Fernandes PB. Susceptibility of *Campylobacter pylori* to macrolides and fluoroquinolones. J Antimicrob Chemother. 1988;22:631–6.
26. DeCross AJ, Marshall BJ, McCallum RW, Hoffman SR, Barrett LJ, Guerrant RL. Metronidazole susceptibility testing for *Helicobacter pylori*: comparison of disk, broth, and agar dilution methods and their clinical relevance. J Clin Microbiol. 1993;31:1971–4.
27. Hachem CY, Clarridge JE, Reddy R, Flamm R, Evans DG, Tanaka SK, et al. Antimicrobial susceptibility testing of *Helicobacter pylori*: comparison of E test, broth microdilution, and disk diffusion for ampicillin, clarithromycin, and metronidazole. Diagn Microbiol Infect Dis. 1996;24:37–41.
28. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; twelfth informational supplement. Approved guideline M100-S12. Wayne: National Committee for Clinical Laboratory Standards; 2002. p. 117.
29. Glupczynski Y, Broutet N, Cantagrel A, Andersen LP, Alarcon T, López-Brea M, et al. Comparison of the E test and agar dilution method for antimicrobial susceptibility testing of *Helicobacter pylori*. Eur J Clin Microbiol Infect Dis. 2002;21:549–52.

11

Urea Breath Test

Yong Hwan Kwon

Abstract

The urea breath test (UBT) is a noninvasive, simple, and safe test that provides excellent accuracy both for the initial diagnosis of *Helicobacter pylori* (*H. pylori*) infection (high sensitivity: 90–100% and specificity: 90–100%). This test is based on the organism's urease activity, which liberates carbon dioxide (CO_2) from urea and produces ammonia to buffer its acidic environment. Ingestion of labeled urea results in the production of labeled CO_2, which then can be detected in the breath. Urea can be labeled with two different isotopes, ^{14}C (the radioactive isotope) or ^{13}C (the nonradioactive stable isotope). The methodology of the UBT has been changed including the dose of labeled urea used, the type of test meal, the time of breath collection, the cutoff values, and the equipment adapted to measure isotope enrichment. This test is also used to ascertain the eradication, and it is recommended for the "test-and-treat" strategy. Even though there is no definitive standardization of this test and the quantitative results may be influenced by sex, age, body mass index; especially obesity, smoking, gastric atrophy and intestinal metaplasia, and even socioeconomic status, UBT is regarded as a gold standard noninvasive method for *H. pylori* diagnosis.

Keywords

Diagnosis · Eradication · *Helicobacter pylori*
Urea breath test

11.1 Introduction

For detecting *Helicobacter pylori* (*H. pylori*) infection, several invasive and noninvasive diagnostic methods have been developed. Although histology of gastric biopsy specimens is regarded as a gold standard for the detection of *H. pylori*, it has the potential for sampling error because of the heterogeneous localization of *H. pylori* in the stomach [1]. A serological examination is a noninvasive and convenient method, but it does not reflect the current status of *H. pylori* infection [1]. To overcome this limitation of serology and invasive methods, the urea breath test (UBT) using ^{13}C or ^{14}C-labeled urea has been developed [2–5]. In 1987, the ^{13}C-UBT was introduced for the detection of *H. pylori* infection [6]. The UBT is a noninvasive test based on the potent urease activity of *H. pylori* in the gastric mucosa. This test has been widely used because it was reported to have a sensitivity and specificity greater than 90% for detecting *H. pylori* infection and to be a more convenient and safer method for the patients [3, 7]. Therefore, the ^{13}C-UBT has become the

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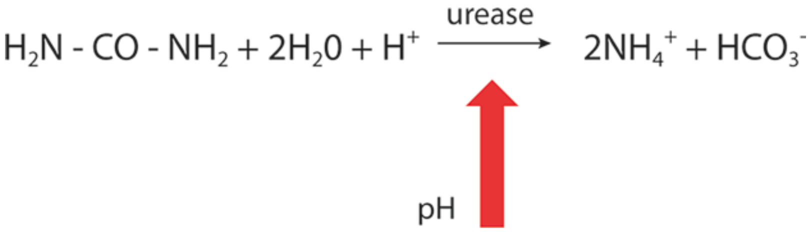
gold standard for the diagnosis of *H. pylori* infection [3–7]. Breath tests are used in gastroenterology practice to study pathophysiological and metabolic processes in an indirect way [8]. These tests have been used to measure gastric emptying, small-bowel bacterial overgrowth, exocrine pancreatic function, liver metabolic capacity, and finally, the presence of *H. pylori* in the stomach [8]. This test has become, during recent years, increasingly popular in clinical practice both for screening patients before endoscopy and for assessing the success of *H. pylori* eradication therapies. The UBT is simple, innocuous, easy to repeat, and highly accurate [3]. It is particularly suitable in all clinical conditions where endoscopy is not strictly necessary, and to check the success of eradication regimens.

11.2 Principle of the Urea Breath Test

The $^{13/14}$C-UBT exploits the copious amounts of urease produced by *H. pylori*, which hydrolyzes urea to form ammonia and soluble carbon dioxide, which is expired in the exhaled breath [9]. Labeling of urea with either isotope allows the $^{13/14}CO_2$ to be detected in the expired breath. Isotope enrichment can be measured by various methods in breath samples collected at appropriate times. A particular biochemical property of *H. pylori* facilitates the use of the ^{13}/^{14}C-UBT to diagnose the presence of the organism in gastric or duodenal mucosa. This microbe produces relatively high concentrations of urease, an enzyme that hydrolyzes urea into the alkaline buffer ammonium and bicarbonate (Fig. 11.1). Bicarbonate generated in gastric mucosa enters the bloodstream, is transported to the lungs, and is rapidly excreted from the lungs as volatile carbon dioxide. In the ^{13}/^{14}C-UBT, a capsule of urea tagged with radioactive carbon is administered orally to a patient; the patient's exhaled carbon dioxide is then collected in airtight balloons. Detection of elevated quantities of exhaled carbon dioxide tagged with $^{13/14}CO_2$ indicates marked bacterial urease activity in the patient's stomach. Production of ammonium and bicarbonate could allow local buffering of highly acidic (pH 1.5) gastric secretions and confer on *H. pylori* its unique microbiologic ability to survive in the hostile gastric lumen [10]. Vigorous urease activity, then, might be vital to the organism's ecologic niche, and the elevated $^{13/14}CO_2$ measurements of exhaled carbon dioxide labeled with $^{13/14}CO_2$ from intense urea metabolism is a biologically plausible indirect measure of *H. pylori* presence [11] (Fig. 11.2). Since UBT had been introduced in clinical settings, the ^{13}C-labeled UBT has garnered significant interest, and thus, it is recommended by several national and international guidelines and several expert consensuses reports over ^{14}C-labeled urea because ^{13}C is a stable, nonradioactive carbon isotope with a natural abundance of approximately 1% among all carbon isotopes [12]. However, the measurement of ^{13}C-labeled carbon dioxide by conventional methods is more expensive, and it requires skilled personnel. Therefore, other alternatives with less expensive methods, such as infrared spectroscope and laser-assisted ratio analyzer, have been developed to measure the ^{13}C-labeled carbon dioxide activity.



$$H_2N - CO - NH_2 + 2H_20 + H^+ \xrightarrow{\text{urease}} 2NH_4^+ + HCO_3^-$$

Fig. 11.1 Reaction of urea hydrolysis in which urea is broken down to ammonia and carbon dioxide by *H. pylori*

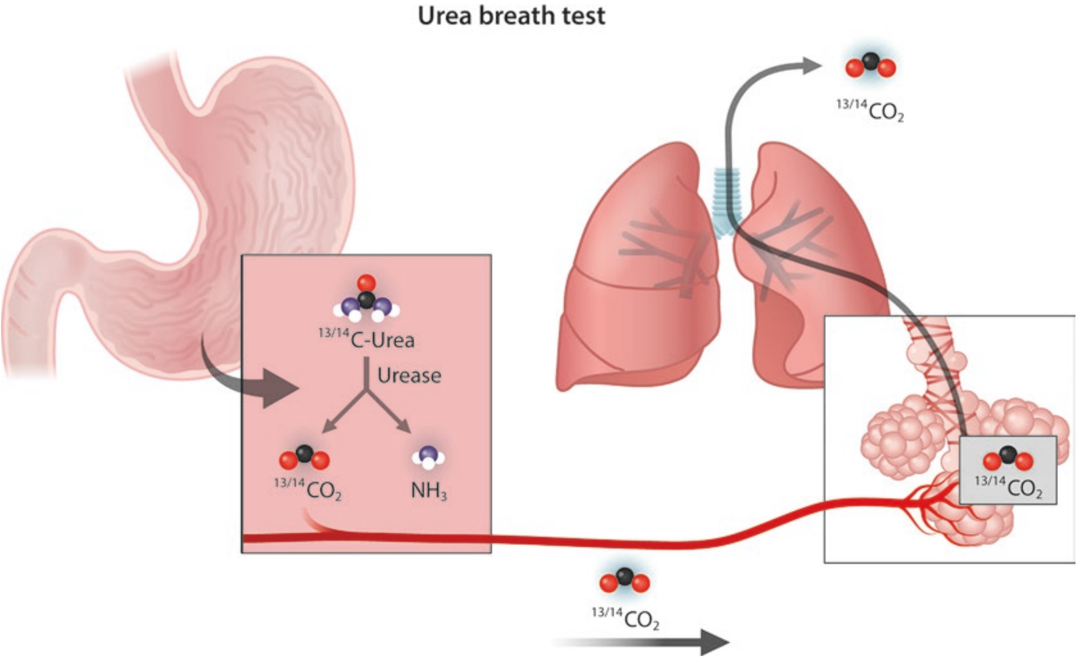


Fig. 11.2 Principles of the urea breath test. On an empty stomach, the patient swallows carbon-labeled urea. In the presence of *H. pylori*, the labeled urea is metabolized into carbon dioxide and ammonia by the enzyme urease, produced by *H. pylori*. The carbon dioxide produced diffuses into the blood vessels, and from there, it is transported as bicarbonate into the lungs and expelled as CO_2 with exhaled air to be captured during sampling. A positive answer offers conclusive evidence that the patient is infected with *H. pylori*. In the absence of *H. pylori*, the administered urea is absorbed from the gastrointestinal tract and subsequently voided (Adapted from http://www.kibion.se/scientific/info-diagnostic-methods/about-breath-tests/)

11.3 Urea Substrate and Measuring Equipment of the Urea Breath Test

While the first UBT was performed with 350 mg of urea [6], UBT may be performed with relatively low doses of urea: 100 mg, 75 mg (mean sensitivity and specificity: 97%), or even 50 mg (mean sensitivity and specificity: 98% [13, 14]. With the most widely used protocols (100 mg without citric acid and 75 mg with citric acid), excellent accuracy is obtained when breath samples are collected as early as 10–15 min after urea ingestion with a mean sensitivity and specificity of almost 100% [15]. Urease-producing oropharyngeal bacteria may theoretically cause false-positive UBT results. As previously mentioned, to overcome this problem, some investigators have encapsulated the urea or administered it as a tablet [14, 16–19]. Urea can be labeled with two different carbon isotopes: ^{14}C and ^{13}C. The main difference between them is that the former is radioactive, whereas the latter is stable [20]. The advantages of using ^{14}C-urea are that it is cheap and so rapid that administering ^{14}C-urea in a gelatin capsule allows for an accurate response to be obtained from a single 10-min breath sample [13, 21], and it does not require any test meal. However, although the dose of ^{14}C has become progressively smaller and the test can now be performed with 1 μCi, which is equal to the natural background radiation received in 1 day [9, 21], the main problems are still the availability of a nuclear medicine department or centers licensed for storage and disposal of radioactive substrates, shipping difficulties, and the copious amounts of labeled tracer needed to perform large-scale epidemiological studies. In contrast, ^{13}C is a nonradioactive isotope that can be used safely for repeated testing, which is frequently required in clinical practice, and for detecting *H. pylori* infection in children and women of childbearing age [20]. However, the major drawbacks of ^{13}C-urea are the higher cost compared with ^{14}C-urea and the need for expensive mass spectrometry, which is the most preferable device for measuring ^{13}C enrichment in breath samples of subjects infected with *H. pylori* [20].

After hydrolysis of ^{13}C-labeled urea by *H. pylori* urease in the stomach and the formation of $^{13}CO_2$, which is transported to the lungs, ^{13}C must be detected in exhaled air by specific measuring equipment. ^{13}C is measured as the $^{13}CO_2$:$^{12}CO_2$ isotope ratio and is expressed as delta over baseline (DOB, δ) per mil (‰) (Fig. 11.3) with respect to the international reference standard represented by the Pee Dee Belemnite limestone [20]: isotope ratio mass spectrometer (IRMS), nondispersive isotope selective infrared spectroscope (NDIRS), and laser-assisted ratio analyzer (LARA) equipment [22] (Table 11.1). The advantage of IRMS is that it processes the highest number of breath samples and works in an automated manner. However, it is the most expensive and requires the longest analysis time. NDIRS does not require helium as the carrier gas and has the lowest weight and price, but it can analyze only a small number of samples and so does not need multitasking software. The LARA system has the quickest analysis time; the other characteristics are average compared with the other two machines, especially with regard to the number of samples it can process and the cost. Integrated bar code readers and the use of multitasking programs have made running IRMS instruments much easier; however, they do require more maintenance than NDIRS and LARA systems. IRMS and LARA are more suitable for gastroenterological centers requiring a large quantity of automated analysis, whereas NDIRS is more suitable for small laboratories in which the daily number of assays is not high. In this light, a small, new, cheap, infrared device for the examination of only two breath samples has been developed to be used exclusively in the doctor's office [20].

$$\delta^{13}C = \left(\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{sample}} - 1 \right) * 1000‰$$

Fig. 11.3 Definition of Delta over baseline (DOB, δ-value). International standard, the Pee Dee Belemnite (PDB)

Table 11.1 Comparison of the main characteristics of three different types of equipment for measuring the $^{13}CO_2$ to $^{12}CO_2$ ratio in breath samples

Characteristics of the instruments	Mass spectrometer (IRMS)	Infrared spectrometer (NDIRS)	Laser-assisted ratio analyzer (LARA)
Weight	90 kg	12 kg	350 kg
Reference gas	Necessary	Enclosed	Necessary
Carrier gas	Helium supply	None	None
Analysis time	120 s	90 s	60 s
Automation	For 220 samples	For 16 samples	For 60 samples
Breath sample	10 ml glass tube	1200 breath bag	12 ml plastic tube
Sample mailing	Favorable	Not practicable	Favorable
Multitasking software	Commonly available	Unnecessary	Planned
Technical expertise	Easy to operate	Easy to operate	Easy to operate
Reported reliability	90–100%	90–98%	Limited experience

Adapted from Savarino et al. [20]

11.4 Test Meal

The original ^{13}C-UBT employed a test meal designed to slow gastric emptying and to maximize the distribution of the substrate within the stomach so as to increase the area and time of contact between the bacteria and the substrate [6]. Test meals containing fat are usually chosen due to the well-known ability of this nutrient to delay gastric emptying. Recently, citric acid, which acts by lowering duodenal pH, which in turn reduces antral motility and relaxes the gastric fundus has been shown to be an optimal test meal [20]. Citric acid test meals have also been shown to enhance the amount of urea hydrolysis compared with traditional nutrient meals without increasing the rate of false-positive results [23–25]. The citric acid test meal also produced a more rapid increase in labeled CO_2 in the breath, suggesting that a post dose breath collection period as short as 10 min might be used for the categorization of *H. pylori* status [24]. The effect was not due to pH as neither pentagastrin stimulated an increase in acidity nor administration of ascorbic acid at a similar pH showed an increase in urea hydrolysis equal to that seen with citric acid [24, 26]. In addition to citric acid, other test drinks like orange or apple juice have been used because of a better taste. Several studies have included orange juice as a test meal, but the diagnostic accuracy of the UBT with either orange juice or citric acid solution debated [17, 27–29]. However, some authors have also evaluated and demonstrated equivalence results without using citric acid (or any other test meal) in UBT [15, 30–36]. A study evaluated the effect of citric acid on the accuracy of UBT. In this prospective study, 1207 *H. pylori*-positive patients received UBT after a successful eradication treatment. They were then divided into two groups: one received a citric acid meal whereas the other one did not. The UBT values after the use of citric acid were significantly higher compared to the control group. In addition, 122 patients were also evaluated by endoscopic biopsy methods. Compared to invasive tests, there was no significant difference in the UBT performance between the two groups, even if gastric atrophy and intestinal metaplasia decreased UBT accuracy [37].

11.5 Time of Breath Collection

Breath samples of UBT were collected before (baseline) and after substrate ingestion. In the original protocol by Graham et al. [6], breath samples were collected every 10 min for 3 h. Subsequently, the test has been progressively simplified using the protocol by measuring $^{13}CO_2$ at 20–30 min after urea ingestion. It has been shown that sampling too early may produce false-positive results because of the urease activity of oral bacteria [6, 38]. Conversely, sampling too late may produce false-negative results because of the emptying of urea from the stomach. Accuracy with these simplified protocols was very high, with both sensitivity and specificity of 97%. In conclusion, when UBT is performed following the most widely used protocol (i.e., with citric acid and 75 mg of urea solution), excellent accuracy results (sensitivity of 99% and specificity of 98%) are obtained when breath samples are collected as early as 10–15 min after urea ingestion [3].

11.6 Diagnostic Accuracy and Appropriate Cutoff Point of the Urea Breath Test

The sensitivity and specificity of the UBT were known to exceed 90% in previous studies. A recent meta-analysis, which evaluated the accuracy of UBT, showed that the sensitivity was 97% (95% CI, 96–98%), and specificity was 96% (95% CI, 95–97%), with significant heterogeneity between studies [39]. Furthermore, a Cochrane review of noninvasive diagnostic tests for *H. pylori* found that UBT had high diagnostic accuracy, while serology and stool antigen tests had lower accuracy in detecting *H. pylori* infection. In this systemic review, the authors showed that, on average, if 1000 people are tested, there will be 46 people without *H. pylori* who will be misdiagnosed as having *H. pylori*. Moreover, there will be 30, 42, 86, and 89 people with *H. pylori* infection for whom the diagnosis of *H. pylori* infection will be missed by ^{13}C-UBT, ^{14}C-UBT, serology, and stool antigen test, respectively [40].

However, the precise choice of the cutoff point to define whether the UBT is positive or negative still represents a controversial issue. A unique and generally proposed cutoff level is not possible, because it has to be adapted to different factors, such as the test meal, the dose, and the type of urea, or the pre/posttreatment setting [3, 13–15, 17–19, 23, 25, 28, 32, 34, 36, 41–59] (Table 11.2). Previously, Logan et al. [9] suggested that the cutoff value for the ^{13}C-UBT was 5.0‰ based on the normal distribution of excess $\delta^{13}CO_2$ values for *H. pylori*-negative subjects who have never been infected. Later studies reported that the cut-off value of the ^{13}C-UBT could be lowered from 3.5‰ to 3.0‰ [60], and the cutoff value was even lower at about 2.5‰ with the use of lower doses of urea, and this value was generally accepted as the best cutoff point [14, 15, 17, 23, 25, 28, 32, 34, 41–46, 61, 62]. Several studies had set up the cutoff point between 1.3‰ and 7.4‰ with high sensitivity and specificity of the ^{13}C-UBT after *H. pylori* eradication [2, 3]. However, many reports suggested that a ^{13}C-UBT value between 2.0‰ and 5.0‰ could fall into the "grey zone," in which the results are inconclusive due to many variant factors affecting the ^{13}C-UBT [2, 60]. By contrast, a recent Spanish study reported that the ^{13}C-UBT (UBit 100 mg, Otsuka Pharmaceutical Europe) was associated with very low specificity (60%) with the previous cutoff value (2.5‰) [54]. They applied for a higher cutoff value (8.5‰) of the ^{13}C-UBT and considerably acquired reliability in the healthy volunteers, although the sensitivity and specificity remained near 90% when UBiTkit 100 mg was applied for this test. In a Korean retrospective study [59], the prevalence of the false negative result in the ^{13}C-UBT value in the 2.0‰ to 2.5‰ range was very low (1.5%). In contrast, the prevalence of the false positive rate in each value of the ^{13}C-UBT between 2.5‰ and 10.0‰ range was from 6.7% to 77.3%. These occurred for results that were near the cutoff value, such as a DOB between the cutoff value. The problem is most common for patients with atrophic gastritis, likely owing to the presence of non–*H. pylori* urease–containing organisms [26, 59]. Thus, the addition of citric acid, which suppresses the delta over baseline value in uninfected individuals and increases the value in subjects with *H. pylori* infection, should reduce the problem, but this strategy requires direct testing. False-positive results lead to the erroneous conclusion that antibiotic treatment failed. This is one cause of what appears to be multiple treatment failures [63]. False negative UBT results have been reported to occur in as many as 40% of individuals taking proton pump inhibitors [17, 62]. One is related to the effect of the proton pump inhibitor on intragastric pH, which could make the intragastric environment unattractive for *H. pylori* and thus indirectly reduce the bacterial load. Alternatively, the pH could be increased sufficiently to close the postulated urea channel, UreI, and thus reduce urea's access to *H. pylori* urease [2]. Besides the effect on pH, the known direct anti–*H. pylori* antimicrobial effect of proton pump inhibitors could result in a direct reduction in bacterial load below the critical threshold of urea hydrolysis required for a positive test. Graham et al. [62] reported that acidification of the stomach did not prevent false negative UBT results. Three days are likely the minimum delay from stopping PPI until one should perform a test for active infection. A delay of 14 days is preferred [20]. Moreover, the UBT has shown heterogeneous accuracy in the pediatric population, especially in young children, with values of sensitivity and specificity ranging from 75% to 100%, before and after treatment (using several protocols), despite being a simple and safe noninvasive test in children older than 6 years old [64]. Although several modifications have been proposed since the original description by Graham of the ^{13}C-UBT to diagnose *H. pylori* infection [6], in children, performance criteria are not yet sufficiently established [65].

Table 11.2 Diagnostic accuracy of the ^{13}C-urea breath test

Author	Indication	Urea dose (mg)	Test meal	Cutoff point (δ‰)	Sensitivity (%)	Specificity (%)
Braden et al. [41]	Pre	75	None	5	99	100
Dominguez et al. [25]	Pre	75	Citric acid	4	100	100
Gatta et al. [14, 17]	Pre/post	50, 75, 100	Citric acid	1.6–7.4	100	100
Gisbert et al. [3, 32, 42]	Pre/post	100	None or citric acid	3.3–4.6	100	100
Hamlet et al. [13, 43]	Pre/post	100	Citric acid	1.8–4.6	95–100	97–100
Kato et al. [44]	Pre/post	100	None	2.5–3.5	67–100	96–100
Klein et al. [23]	Pre/post	125, 250	Milk	2.4–4.2	100	98–100
Leodolter et al. [28, 45]	Pre/post	75	Citric acid	4	92–100	98–100
Liao et al. [46]	Pre	50	Milk	2.5	99	95–97
Malaty et al. [15]	Pre	125	None	2.4	96	100
Miwa et al. [34]	Pre/post	100	None	5	96	97
Ng et al. [47]	Pre	75	None or citric acid	3.5–5	93–97	94–97
Savarino et al. [48]	Pre/post	75	Citric acid	5	98–100	97–100
Sheu et al. [50, 51]	Pre/post	50, 100	None or citric acid	2.5–4	94–97	95–99
Wong et al. [18, 19]	Pre/post	50, 75	None or citric acid	1.2–7	96–100	90–100
Gomollon et al. [52]	Pre	100	Citric acid	5	97.3	100
Urita et al. [53]	Pre	100	None	2.5	97.7	94
Calvet et al. [54]	Pre	100	None or citric acid	8.5	99.2	60.5
Peng et al. [55, 56]	Pre	50, 100	None	4.8–5.0	100	95.7–100
Wardi et al. [57]	Pre	75	4.5 g citric acid-based powder	5	64	91
Li et al. [58]	Pre	75	None	3.8	94.5	92.9
Kwon et al. [59]	Pre	100	None or citric acid	2.5	91.7	81.3

Pre Before eradication treatment, *Post* After treatment to assess *Helicobacter pylori* eradication success

Few studies using UBT have been performed in patients subjected to a partial gastrectomy, a specific group in which the identification of *H. pylori* infection is mostly relevant. After partial gastrectomy, the gastric anatomy is altered, and the test urea might be expected to pass through the stomach faster, giving different reactant percentages in reaction time, or the ^{13}C-UBT results could be influenced by bile acid reflux. In a hypochlorhydria state of the remnant stomach, it accelerates the colonization and the overgrowth of non*H. pylori* urease-positive bacteria (*Streptococcus*, *Staphylococcus*, *Gardnerella*, *Lactococcus,* and *Enterococcus*) [66], and elevation of intragastric pH removes the neutralizing action of hydrochloric acid on local ammonia production by *H. pylori* urease, leading to the ultimate death of the bacterium as a result of overalkalization [67–69]. Several reports have shown that ^{13}C-UBT provides lower diagnostic accuracy when using histology as a reference in the remnant stomach after partial gastrectomy, and recent meta-analyses have shown that people without a history of gastrectomy and those who have not recently had antibiotics or proton, pump inhibitors, urea breath tests had high diagnostic accuracy while serology and stool antigen tests were less accurate for diagnosis of *Helicobacter pylori* infection [40, 70, 71].

11.7 Conclusion

Since the discovery of *H. pylori* in 1983, many different methods have been used for the detection of *H. pylori* infection, either noninvasive or invasive. The ^{13}C-UBT is the ideal test for those in whom endoscopy is not required because it offers the combination of simplicity, accuracy, absence of exposure to radioactivity, and reliability. However, definitive standardization of the protocol for UBT has to be evaluated.

References

1. Morris A, Ali MR, Brown P, Lane M, Patton K. *Campylobacter pylori* infection in biopsy specimens of gastric antrum: laboratory diagnosis and estimation of sampling error. J Clin Pathol. 1989;42:727–32.
2. Graham DY, Klein PD. Accurate diagnosis of *Helicobacter pylori*. ^{13}C-urea breath test. Gastroenterol Clin N Am. 2000;29:885–93.
3. Gisbert JP, Pajares JM. Review article: ^{13}C-urea breath test in the diagnosis of *Helicobacter pylori* infection—a critical review. Aliment Pharmacol Ther. 2004;20:1001–17.
4. Bazzoli F, Zagari M, Fossi S, Pozzato P, Ricciardiello L, Mwangemi C, et al. Urea breath tests for the detection of *Helicobacter pylori* infection. Helicobacter. 1997;2 (Suppl 1):S34–7.
5. Thomas JE. ^{13}C urea breath test. Gut. 1998;43 (Suppl 3):S7–12.
6. Graham DY, Klein PD, Evans DJ Jr, Evans DG, Alpert LC, Opekun AR, et al. *Campylobacter pylori* detected noninvasively by the ^{13}C-urea breath test. Lancet. 1987;1:1174–7.
7. Cutler AF, Havstad S, Ma CK, Blaser MJ, Perez-Perez GI, Schubert TT. Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection. Gastroenterology. 1995;109:136–41.
8. Lacroix M, Mosora F, Pontus M, Lefebvre P, Luyckz A, Lopez-Habib G. Glucose naturally labeled with carbon-13: use for metabolic studies in man. Science. 1973;181:445–6.
9. Logan RP. Urea breath tests in the management of *Helicobacter pylori* infection. Gut. 1998;43:S47–50.
10. Dunn BE. Pathogenic mechanisms of *Helicobacter pylori*. Gastroenterol Clin N Am. 1993;22:43–57.
11. Felz MW, Burke GJ, Schuman BM. Breath test diagnosis of *Helicobacter pylori* in peptic ulcer disease: a noninvasive primary care option. J Am Board Fam Pract. 1997;10:385–9.
12. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou J-M, Schulz C, et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
13. Hamlet AK, Erlandsson KI, Olbe L, Svennerholm AM, Backman VE, Pettersson AB. A simple, rapid, and highly reliable capsule-based ^{14}C urea breath test for diagnosis of *Helicobacter pylori* infection. Scand J Gastroenterol. 1995;30:1058–63.
14. Gatta L, Vakil N, Ricci C, Osborn JF, Tampieri A, Perna F, et al. A rapid, low-dose, ^{13}C-urea tablet for the detection of *Helicobacter pylori* infection before and after treatment. Aliment Pharmacol Ther. 2003;17:793–8.
15. Malaty HM, el-Zimaity HM, Genta RM, Klein PD, Graham DY. Twenty-minute fasting version of the US ^{13}C-urea breath test for the diagnosis of *H. pylori* infection. Helicobacter. 1996;1:165–7.
16. Bielanski W, Konturek SJ. New approach to ^{13}C-urea breath test: capsule-based modification with low-dose of ^{13}C-urea in the diagnosis of *Helicobacter pylori* infection. J Physiol Pharmacol. 1996;47:545–53.
17. Gatta L, Vakil N, Ricci C, Osborn JF, Tampieri A, Perna F, et al. Effect of proton pump inhibitors and antacid therapy on ^{13}C urea breath tests and stool test for *Helicobacter pylori* infection. Am J Gastroenterol. 2004;99:823–9.

18. Wong WM, Wong BC, Li TM, Wong KW, Cheung KL, Fung FM, et al. Twenty-minute 50 mg ^{13}C-urea breath test without test meal for the diagnosis of *Helicobacter pylori* infection in Chinese. Aliment Pharmacol Ther. 2001;15:1499–504.
19. Wong WM, Lam SK, Lai KC, Chu KM, Xia HH, Wong KW, et al. A rapid-release 50-mg tablet-based ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2003;17:253–7.
20. Savarino V, Vigneri S, Celle G. The ^{13}C urea breath test in the diagnosis of *Helicobacter pylori* infection. Gut. 1999;45 (Suppl 1):I18–22.
21. Peura DA, Pambianco DJ, Dye KR, Lind C, Frierson HF, Hoffman SR, et al. Microdose ^{14}C-urea breath test offers diagnosis of *Helicobacter pylori* in 10 minutes. Am J Gastroenterol. 1996;91:233–8.
22. Parente F, Bianchi PG. The ^{13}C-urea breath test for non-invasive diagnosis of *Helicobacter pylori* infection: which procedure and which measuring equipment? Eur J Gastroenterol Hepatol. 2001;13:803–6.
23. Klein PD, Malaty HM, Martin RF, Graham KS, Genta RM, Graham DY. Noninvasive detection of *Helicobacter pylori* infection in clinical practice: the ^{13}C urea breath test. Am J Gastroenterol. 1996;91:690–4.
24. Graham DY, Runke D, Anderson SY, Malaty HM, Klein PD. Citric acid as the test meal for the ^{13}C-urea breath test. Am J Gastroenterol. 1999;94:1214–7.
25. Domínguez-Muñoz JE, Leodolter A, Sauerbruch T, Malfertheiner P. A citric acid solution is an optimal test drink in the ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection. Gut. 1997;40:459–62.
26. Graham DY, Malaty HM, Cole RA, Martin RF, Klein PD. Simplified ^{13}C-urea breath test for detection of *Helicobacter pylori* infection. Am J Gastroenterol. 2001;96:1741–5.
27. Coelho LG, Reber M, Passos MC, Aguiar RO, Casaes PE, Bueno ML, et al. Application of isotope-selective non-dispersive infrared spectrometry for the evaluation of the ^{13}C-urea breath test: comparison with three concordant methods. Braz J Med Biol Res. 1999;32:1493–7.
28. Leodolter A, Domínguez-Muñoz JE, Von Arnim U, Malfertheiner P. Citric acid or orange juice for the ^{13}C-urea breath test: the impact of pH and gastric emptying. Aliment Pharmacol Ther. 1999;13:1057–62.
29. Labenz J, Bärsch G, Peitz U, Aygen S, Hennemann O, Tillenburg B, et al. Validity of a novel biopsy urease test (HUT) and a simplified ^{13}C-urea breath test for diagnosis of *Helicobacter pylori* infection and estimation of the severity of gastritis. Digestion. 1996;57:391–7.
30. Chen TS, Chang FY, Chen PC, Huang TW, Ou JT, Tsai MH, et al. Simplified ^{13}C-urea breath test with a new infrared spectrometer for diagnosis of *Helicobacter pylori* infection. J Gastroenterol Hepatol. 2003;18:1237–43.
31. Perri F, Maes B, Geypens B, Ghoos Y, Hiele M, Rutgeerts P. The influence of isolated doses of drugs, feeding and colonic bacterial ureolysis on urea breath test results. Aliment Pharmacol Ther. 1995;9:705–9.
32. Gisbert JP, Vazquez MA, Jimenez I, Cruzado AI, Carpio D, Del Castillo E, et al. ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection before treatment: is citric acid necessary? Dig Liver Dis. 2000;32:20–4.
33. Oksanen A, Bergström M, Sjöstedt S, Gad A, Hammarlund B, Seensalu R. Accurate detection of *Helicobacter pylori* infection with a simplified ^{13}C urea breath test. Scand J Clin Lab Invest. 1997;57:689–94.
34. Miwa H, Murai T, Ohkura R, Nagahara A, Watanabe H, Terai T, et al. Usefulness of the ^{13}C-urea breath test for detection of *Helicobacter pylori* infection in fasting patients. J Gastroenterol Hepatol. 1998;13:1039–43.
35. Casellas F, Lopez J, Borruel N, Saperas E, Vergara M, de Torres I, et al. The impact of delaying gastric emptying by either meal substrate or drug on the ^{13}C-urea breath test. Am J Gastroenterol. 1999;94:369–73.
36. Wong WM, Wong BC, Wong KW, Fung FM, Lai KC, Hu WH, et al. ^{13}C-urea breath test without a test meal is highly accurate for the detection of *Helicobacter pylori* infection in Chinese. Aliment Pharmacol Ther. 2000;14:1353–8.
37. Kwon YH, Kim N, Yoon H, Shin CM, Park YS, Lee DH. Effect of citric acid on accuracy of ^{13}C-urea breath test after *Helicobacter pylori* eradication therapy in a region with a high prevalence of atrophic gastritis. Gut Liver. 2019;13:506–14.
38. Rowland M, Lambert I, Gormally S, Daly LE, Thomas JE, Hetherington C, et al. Carbon 13-labeled urea breath test for the diagnosis of *Helicobacter pylori* infection in children. J Pediatr. 1997;131:815–20.
39. Abd Rahim MA, Johani FH, Shah SA, Hassan MR, Abdul Manaf MR. ^{13}C-urea breath test accuracy for *Helicobacter pylori* infection in the Asian population: a meta-analysis. Ann Glob Health. 2019;85:110.
40. Best LM, Takwoingi Y, Siddique S, Selladurai A, Gandhi A, Low B, et al. Non-invasive diagnostic tests for *Helicobacter pylori* infection. Cochrane Database Syst Rev. 2018;3:Cd012080.
41. Braden B, Duan LP, Caspary WF, Lembcke B. More convenient ^{13}C-urea breath test modifications still meet the criteria for valid diagnosis of *Helicobacter pylori* infection. Z Gastroenterol. 1994;32:198–202.
42. Gisbert JP, Ducons J, Gomollón F, Domínguez-Muñoz JE, Borda F, Miño G, et al. Validation of the ^{13}C-urea breath test for the initial diagnosis of *Helicobacter pylori* infection and to confirm eradication after treatment. Rev Esp Enferm Dig. 2003;95:121–6.
43. Hamlet A, Stage L, Lönroth H, Cahlin C, Nyström C, Pettersson A. A novel tablet-based ^{13}C urea breath test for *Helicobacter pylori* with enhanced performance during acid suppression therapy. Scand J Gastroenterol. 1999;34:367–74.
44. Kato M, Asaka M, Kudo T, Katagiri M, Komatsu Y, Sato F, et al. Ten minute ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection. J Gastroenterol. 1998;33 (Suppl 10):40–3.

45. Leodolter A, Domínguez-Muñoz JE, von Arnim U, Kahl S, Peitz U, Malfertheiner P. Validity of a modified ^{13}C-urea breath test for pre- and posttreatment diagnosis of *Helicobacter pylori* infection in the routine clinical setting. Am J Gastroenterol. 1999;94:2100–4.
46. Liao CC, Lee CL, Chiang TC, Lee SC, Huang SH, Tu TC, et al. The ^{13}C-urea breath test to detect *Helicobacter pylori* infection: a validated simple methodology with 50 mg ^{13}C-urea. Aliment Pharmacol Ther. 2002;16:787–92.
47. Ng FH, Lai KC, Wong BC, Wong WM, Wong SY, Chow KC, et al. ^{13}C-urea breath test without prior fasting and without test meal is accurate for the detection of *Helicobacter pylori* infection in Chinese. J Gastroenterol Hepatol. 2002;17:834–8.
48. Savarino V, Mela GS, Zentilin P, Bisso G, Pivari M, Mansi C, et al. Comparison of isotope ratio mass spectrometry and nondispersive isotope-selective infrared spectroscopy for ^{13}C-urea breath test. Am J Gastroenterol. 1999;94:1203–8.
49. Savarino V, Landi F, Dulbecco P, Ricci C, Tessieri L, Biagini R, et al. Isotope ratio mass spectrometry (IRMS) versus laser-assisted ratio analyzer (LARA): a comparative study using two doses of. Dig Dis Sci. 2000;45:2168–74.
50. Sheu BS, Lee SC, Yang HB, Wu HW, Wu CS, Lin XZ, et al. Lower-dose ^{13}C-urea breath test to detect *Helicobacter pylori* infection-comparison between infrared spectrometer and mass spectrometry analysis. Aliment Pharmacol Ther. 2000;14:1359–63.
51. Sheu BS, Lee SC, Yang HB, Kuo AW, Wang YL, Shiesh SC, et al. Selection of lower cutoff point of ^{13}C urea breath test is helpful to monitor *H. pylori* eradication after proton pump inhibitor-based triple therapy. Dig Dis Sci. 2000;45:1330–6.
52. Gomollón F, Ducons JA, Santolaria S, Lera Omiste I, Guirao R, Ferrero M, et al. Breath test is very reliable for diagnosis of *Helicobacter pylori* infection in real clinical practice. Dig Liver Dis. 2003;35:612–8.
53. Urita Y, Hike K, Torii N, Kikuchi Y, Kanda E, Kurakata H, et al. Breath sample collection through the nostril reduces false-positive results of ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection. Dig Liver Dis. 2004;36:661–5.
54. Calvet X, Sánchez-Delgado J, Montserrat A, Lario S, Ramírez-Lázaro MJ, Quesada M, et al. Accuracy of diagnostic tests for *Helicobacter pylori*: a reappraisal. Clin Infect Dis. 2009;48:1385–91.
55. Peng NJ, Lai KH, Lo GH, Hsu PI. Comparison of noninvasive diagnostic tests for *Helicobacter pylori* infection. Med Princ Pract. 2009;18:57–61.
56. Peng NJ, Lai KH, Liu RS, Lee SC, Tsay DG, Lo CC, et al. Capsule ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection. World J Gastroenterol. 2005;11:1361–4.
57. Wardi J, Shalev T, Shevah O, Boaz M, Avni Y, Shirin H. A rapid continuous-real-time ^{13}C-urea breath test for the detection of *Helicobacter pylori* in patients after partial gastrectomy. J Clin Gastroenterol. 2012;46:293–6.
58. Li ZX, Huang LL, Liu C, Formichella L, Zhang Y, Wang YM, et al. Cut-off optimization for ^{13}C-urea breath test in a community-based trial by mathematic, histology and serology approach. Sci Rep. 2017;7:2072.
59. Kwon YH, Kim N, Lee JY, Choi YJ, Yoon K, Hwang JJ, et al. The diagnostic validity of citric acid-free, high dose ^{13}C-urea breath test after *Helicobacter pylori* eradication in Korea. Helicobacter. 2015;20:159–68.
60. Mion F, Rosner G, Rousseau M, Minaire Y. ^{13}C-urea breath test for *Helicobacter pylori*: cut-off point determination by cluster analysis. Clin Sci. 1997;93:3–6.
61. Kopácová M, Bures J, Vorísek V, Konstacký M, Rejchrt S, Zivný P, et al. Comparison of different protocols for ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection in healthy volunteers. Scand J Clin Lab Invest. 2005;65:491–8.
62. Graham DY, Opekun AR, Hammoud F, Yamaoka Y, Reddy R, Osato MS, et al. Studies regarding the mechanism of false negative urea breath tests with proton pump inhibitors. Am J Gastroenterol. 2003;98:1005–9.
63. Graham DY. *Helicobacter pylori* update: gastric cancer, reliable therapy, and possible benefits. Gastroenterology. 2015;148:719–31.e3.
64. Guarner J, Kalach N, Elitsur Y, Koletzko S. *Helicobacter pylori* diagnostic tests in children: review of the literature from 1999 to 2009. Eur J Pediatr. 2010;169:15–25.
65. Machado RS, Patricio FR, Kawakami E. ^{13}C-urea breath test to diagnose *Helicobacter pylori* infection in children aged up to 6 years. Helicobacter. 2004;9:39–45.
66. Brandi G, Biavati B, Calabrese C, Granata M, Nannetti A, Mattarelli P, et al. Urease-positive bacteria other than *Helicobacter pylori* in human gastric juice and mucosa. Am J Gastroenterol. 2006;101:1756–61.
67. Bell NJ, Hunt RH. Progress with proton pump inhibition. Yale J Biol Med. 1992;65:649–57; discussion 689–92.
68. Gurbuz AK, Ozel AM, Narin Y, Yazgan Y, Baloglu H, Demirturk L. Is the remarkable contradiction between histology and ^{14}C urea breath test in the detection of *Helicobacter pylori* due to false-negative histology or false-positive ^{14}C urea breath test? J Int Med Res. 2005;33:632–40.
69. Osaki T, Mabe K, Hanawa T, Kamiya S. Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of *Helicobacter pylori* infection. J Med Microbiol. 2008;57(Pt 7):814–9.
70. Adamopoulos AB, Stergiou GS, Sakizlis GN, Tiniakos DG, Nasothimiou EG, Sioutis DK, et al. Diagnostic value of rapid urease test and urea breath test for *Helicobacter pylori* detection in patients with Billroth II gastrectomy: a prospective controlled trial. Dig Liver Dis. 2009;41:4–8.
71. Sheu BS, Lee SC, Lin PW, Wang ST, Chang YC, Yang HB, et al. Carbon urea breath test is not as accurate as endoscopy to detect *Helicobacter pylori* after gastrectomy. Gastrointest Endosc. 2000;51:670–5.

12

H. pylori Stool Antigen Test

Hye Ran Yang

Abstract

Helicobacter pylori (*H. pylori*) stool antigen (HpSA) test is a reliable noninvasive diagnostic method for the detection of *H. pylori* infection in both adults and children. According to recent data, the HpSA test using an enzyme immunoassay is well validated as a noninvasive test to determine the status of *H. pylori* infection with high diagnostic accuracy before eradication therapy as well as after therapy. The HpSA test using a monoclonal antibody is more accurate than a polyclonal antibody test. The rapid HpSA test is also a reliable test and easy to perform in clinical practice. Recently, a novel monoclonal HpSA test using chemiluminescent immunoassay was newly introduced in practice. When the HpSA test is applied, some factors that can affect the diagnostic accuracy such as drugs, temperature, and gastrointestinal bleeding, and diarrhea as accompanying symptoms should be taken into consideration to interpret the results of the HpSA test.

Keywords

Helicobacter pylori · Stool antigen test
Noninvasive · Diagnosis

12.1 Introduction

Helicobacter pylori (*H. pylori*) stool antigen (HpSA) test is a well-known noninvasive method for diagnosing *H. pylori* infection. Since it was developed and introduced in 1997, the HpSA test has been applied in clinical practice. When compared to other noninvasive diagnostic tests to detect *H. pylori* infection, the HpSA test has some advantages because it can be easily carried out at all ages and is relatively cheap despite its high diagnostic accuracy.

Initially, the HpSA test was based on the enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) methods using polyclonal antibodies to detect *H. pylori* antigens in the feces. Later, HpSA tests using monoclonal antibodies with higher diagnostic accuracy were also developed. Recently, a rapid HpSA test based on immunochromatography methods was also developed and has been validated.

In this chapter, updates on the HpSA tests for the diagnosis of *H. pylori* infection will be reviewed systematically.

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12.2 Diagnostic Accuracy of *H. Pylori* Stool Antigen Test

12.2.1 Diagnostic Accuracy of *H. Pylori* Stool Antigen Test in Untreated Patients

The HpSA test using an EIA method is a noninvasive diagnostic method to identify *H. pylori* infection with high diagnostic accuracy in both adults and children of any age [1]. According to recent studies, its diagnostic accuracy is similar to the urea breath test and higher than both serologic and urine antibody tests [2].

In adults, the diagnostic accuracy of the HpSA test for initial diagnosis of *H. pylori* infection was known to be high with a sensitivity of 89–98% and a specificity of 78–100% [3–5]. Overall diagnostic accuracy of the HpSA test based on EIA, of both polyclonal HpSA and monoclonal HpSA tests, as reported by a systemic review in 2004, revealed a mean sensitivity of 91%, a mean specificity of 93%, a positive predictive value (PPV) of 92%, a negative predictive value (NPV) of 87% [6], a pooled sensitivity of 94%, a specificity of 97%, a positive likelihood ratio (LR+) of 24, and a negative likelihood ratio (LR−) of 0.07 in a meta-analysis reported in 2006 [7]. According to this meta-analysis, the sensitivity of the monoclonal HpSA test (95%) was higher than that of the polyclonal HpSA test (83%) [7].

In children, the diagnostic accuracy of the HpSA test is similarly high as that in adults. A recent meta-analysis study on the HpSA test reported that the HpSA test using ELISA polyclonal antibodies had a sensitivity of 92%, a specificity of 93%, an LR+ of 16.2, and an LR− of 0.09. The monoclonal study also revealed higher diagnostic accuracy in children with a sensitivity of 97%, a specificity of 97%, an LR+ of 29.9, and an LR− of 0.03 [8]. Additionally, another recent meta-analysis in 2014 reported similar diagnostic accuracy of the EIA-based HpSA test in children, reporting an overall pooled sensitivity of 92.1%, a specificity of 94.1%, an LR+ of 17.01, and an LR− of 0.08 [9]. In this meta-analysis performed in children, mean sensitivity and specificity were 92.6% and 93.8%, respectively, for the initial diagnosis of *H. pylori* infection [9].

12.2.2 Diagnostic Accuracy of *H. Pylori* Stool Antigen Test After Eradication

The HpSA test is a useful follow-up method after eradication therapy in both adults and children [10–13]. After the eradication of *H. pylori* infection, diagnostic accuracy is still high with a sensitivity of 70–96% and a specificity of 81–95% [10–13]. In a recent meta-analysis on the accuracy of the HpSA test in children, mean sensitivity and specificity were 80.9% and 97.2%, respectively, with an LR+ of 19.71 and an LR− of 0.171 for the diagnosis of *H. pylori* infection after eradication therapy in children [9]. Recent guidelines from ESPGHAN (European Society for Paediatric Gastroenterology, Hepatology, and Nutrition) and NASPGHAN (North American Society for Paediatric Gastroenterology, Hepatology, and Nutrition) for the diagnosis of *H. pylori* infection in children recommended the HpSA test based on EIA as a reliable diagnostic test to identify the eradication of *H. pylori* infection [14].

When comparing the monoclonal HpSA and the polyclonal HpSA tests, the former is more accurate not only for the initial diagnosis of *H. pylori* infection but also for follow-up after eradication therapy, with a reported pooled sensitivity of 93%, a specificity of 96%, an LR+ of 17, and an LR− of 0.1 [7]. In particular, the HpSA test using EIA monoclonal antibody has higher sensitivity (91%) than the polyclonal test (76%) after eradication of *H. pylori* infection, according to the meta-analysis [7].

12.3 Types of *H. Pylori* Stool Antigen Test

There are two types of conventional HpSA tests based on EIA and immunochromatography assay (ICA) and a recently introduced novel HpSA test based on chemiluminescent immunoassay for the diagnosis of *H. pylori* infection according to the basic techniques. All of them have some advantages in clinical practice because they are inexpensive and noninvasive methods to identify *H. pylori* infection with relatively high diagnostic accuracy. These HpSA tests can be performed just by collecting stool specimens from the patients at any time because stool samples can be stably stored in the freezer before testing.

12.3.1 *H. Pylori* Stool Antigen Test Based on Enzyme Immunoassay

Most HpSA tests used in clinical practice are EIA-based tests using polyclonal or monoclonal antibodies against *H. pylori* antigens from stool specimens, which are coated on microplates and subjected to a sandwich enzyme immunoassay technique [15]. This EIA-based HpSA test is easily performed in all age groups, even in infancy, by collecting stool samples noninvasively without any specialized techniques but requires a spectrophotometer to interpret the test results.

12.3.2 *H. Pylori* Stool Antigen Test Based on Immunochromatography

Rapid HpSA test using monoclonal antibody based on ICA has also been applied for the diagnosis of the infection before and after therapy [13]. The test is very easy to perform and suitable as a one-step in-office test because it takes only 5–15 min to interpret the test results grossly without any expensive equipment or skilled personnel.

However, the diagnostic accuracy of the rapid HpSA test is similar to or a bit lower than the conventional HpSA test [16], with a sensitivity of 88%, a specificity of 93%, an LR+ of 10.6, and an LR− of 0.11, according to a recent meta-analysis study [8]. Therefore, this rapid HpSA test has some limitations in clinical practice despite its convenience.

12.3.3 Novel *H. Pylori* Stool Antigen Tests

A novel monoclonal HpSA test based on chemiluminescent immunoassay was recently developed and applied for the diagnosis of *H. pylori* infection [17–19]. In a prospective cohort study, the diagnostic accuracy of this automated LIAISON® Meridian HpSA test revealed a sensitivity of 95.5% (95% confidence interval (CI), 87.5–99.1%) and a specificity of 97.6% (95% CI, 94.5–99.2%), with a very good agreement with the biopsy-based methods including rapid urease test, history, and culture (Cohen's kappa 0.922) [17]. In another prospective multicenter study, this HpSA test showed the sensitivity, specificity, PPV, NPV, and diagnostic accuracy were 67%, 97%, 86%, 92%, and 91%, respectively, with an AUROC of 0.85, applying the recommended cut-off value of 1.10 [18].

When comparing this novel HpSA test based on chemiluminescence immunoassay with two conventional HpSA tests based on EIA and ICA, there was a high positive percent agreement (EIA, 97.6%; 95% CI, 86.3–100%; ICA, 90.2%; 95% CI, 76.9–96.7%) and a high negative percent agreement between the tests (EIA, 99.5%; 95% CI, 97.0–100%; ICA, 97.1%; 95% CI, 93.7–98.8%), with a very good agreement (EIA, Cohen's kappa 0.971; ICA, Cohen's kappa 0.857) [19].

In addition, a novel HpSA test based on a new technique of immunomagnetic beads with monoclonal antibodies, coupled with a polyclonal antibody conjugating special fluorescently labeled quantum dots, may have potential to detect *H. pylori* more effectively [20].

12.4 *H. Pylori* Stool Antigen Test in Specific Conditions

When applying to the HpSA test clinically, additional attention is required since some factors can affect the test results. Diagnostic accuracy of the HpSA test using loose or watery stool specimens can be relatively lower because *H. pylori* antigens in stool samples are diluted. Thus, HpSA testing is not recommended when the patient has symptoms of diarrhea, especially in the post-treatment setting [21]. However, in another study, it was reported that the false-negative results were not brought on if the dilution was by less than tenfold [22].

When there is upper gastrointestinal bleeding, it has been reported that the diagnostic accuracy of the HpSA test is relatively low [23]. In clinical practice, a monoclonal HpSA test based on ELISA is recommended in such patients [23].

The temperature also affects the diagnostic accuracy of the HpSA test. Although stool specimens can be stored for up to 3 days at 2–8 °C, it is recommended that the test be carried out as soon as possible when the stool is collected. If stool specimens should be saved for a long time, it is good to be stored at −80 °C to maintain its antigenicity [21].

Proton pump inhibitors can also affect the diagnostic accuracy of the test, thus it is recommended to test after a lapse of 1 or 2 weeks after discontinuing the drug [6].

12.5 Conclusion

HpSA test is useful for noninvasive diagnosis of *H. pylori* infection by using stool specimens for testing. HpSA test can be applied to both adults and children, even infants, with excellent diagnostic accuracy before and after eradication therapy of *H. pylori*, although posttreatment accuracy is relatively low after therapy. HpSA test based on monoclonal antibody using ELISA or a novel chemiluminescent immunoassay rather than the polyclonal test may be the more accurate noninvasive method to detect *H. pylori* infection in clinical practice. The rapid HpSA test using monoclonal antibody based on ICA may be a good alternative as an in-office one-step test. Furthermore, newly developed HpSA tests with advanced novel techniques such as immunomagnetic bead capture may be promising in detecting *H. pylori* with better diagnostic yield.

References

1. Konstantopoulos N, Russmann H, Tasch C, Sauerwald T, Demmelmair H, Autenrieth I, et al. Evaluation of the *Helicobacter pylori* stool antigen test (HpSA) for detection of *Helicobacter pylori* infection in children. Am J Gastroenterol. 2001;96:677–83.
2. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of *Helicobacter pylori* infection—the Maastricht IV/Florence consensus report. Gut. 2012;61:646–64.
3. Chang MC, Wu M, Wang HH, Wang HP, Lin JT. *Helicobacter pylori* stool antigen (HpSA) test: a simple accurate and noninvasive test for detection of *Helicobacter pylori* infection. Hepato-Gastroenterology. 1999;46:299–302.
4. Fanti L, Mezzi G, Cavallero A, Gesu G, Bonato C, Masci E. A new simple immunoassay for detecting *Helicobacter pylori* infection: antigen in stool specimens. Digestion. 1999;60:456–60.
5. Ohkura R, Miwa H, Murai T, Nagahara A, Ohta K, Sato K, et al. Usefulness of a novel enzyme immunoassay for the detection of *Helicobacter pylori* in feces. Scand J Gastroenterol. 2000;35:49–53.
6. Gisbert JP, Pajares JM. Stool antigen test for the diagnosis of *Helicobacter pylori* infection: a systematic review. Helicobacter. 2004;9:347–68.
7. Gisbert JP, de la Morena F, Abraira V. Accuracy of monoclonal stool antigen test for the diagnosis of *H. pylori* infection: a systematic review and meta-analysis. Am J Gastroenterol. 2006;101:1921–30.
8. Leal YA, Cedillo-Rivera R, Simón JA, Velázquez JR, Flores LL, Torres J. Utility of stool sample-based tests for the diagnosis of *Helicobacter pylori* infection in children. J Pediatr Gastroenterol Nutr. 2011;52:718–28.
9. Zhou X, Su J, Xu G, Zhang G. Accuracy of stool antigen test for the diagnosis of *Helicobacter pylori* infection in children: a meta-analysis. Clin Res Hepatol Gastroenterol. 2014;38:629–38.
10. Vaira D, Malfertheiner P, Mégraud F, Axon AT, Deltenre M, Gasbarrini G, et al. Noninvasive antigen based assay for assessing *Helicobacter pylori* eradication: a European multicenter study. Am J Gastroenterol. 2000;95:925–9.
11. Ishihara S, Kaji T, Kawamura A, Rumi MA, Sato H, Okuyama T, et al. Diagnostic accuracy of a new

non-invasive enzyme immunoassay for detecting *Helicobacter pylori* in stools after eradication therapy. Aliment Pharmacol Ther. 2000;14:611–4.

12. Gościniak G, Przondo-Mordarska A, Iwańczak B, Blitek A. *Helicobacter pylori* antigens in stool specimens of gastritis children before and after treatment. J Pediatr Gastroenterol Nutr. 2003;36:376–80.
13. Yang HR, Seo JK. *Helicobacter pylori* stool antigen (HpSA) tests in children before and after eradicartion therapy: comparison of rapid immunochromatographic assay and HpSA ELISA. Dig Dis Sci. 2008;53:2053–8.
14. Koletzko S, Jones NL, Goodman KJ, Gold B, Rowland M, Cadranel S, et al. Evidence-based guidelines from ESPGHAN and NASPGHAN for *Helicobacter pylori* infection in children. J Pediatr Gastroenterol Nutr. 2011;53:230–43.
15. Crowley E, Bourke B, Hussey S. How to use *Helicobacter pylori* testing in paediatric practice. Arch Dis Child Educ Pract Ed. 2013;98:18–25.
16. Shimoyama T, Sawaya M, Ishiguro A, Hanabata N, Yoshimura T, Fukuda S. Applicability of a rapid stool antigen test, using monoclonal antibody to catalase, for the management of *Helicobacter pylori* infection. J Gastroenterol. 2011;46:487–91.
17. Opekun AR, Zierold C, Rode A, Blocki F, Fiorini G, Saracino IM, et al. Clinical performance of the automated LIAISON® Meridian *H. pylori* stool antigen test. Biomed Res Int. 2020;2020:7189519.
18. Resina E, Donday M, Martínez-Domínguez S, Laserna-Mendieta EJ, Lanas A, Lucendo A, et al. Evaluation of a new monoclonal chemiluminescent immunoassay stool antigen test for the diagnosis of *Helicobacter pylori* infection: a Spanish multicentre study. J Clin Med. 2022;11:5077.
19. Ignatius R, Berg C, Weiland C, Darmer A, Wenzel T, Lorenz M, et al. Accurate detection of *Helicobacter pylori* antigen in human stool specimens by two novel immunoassays. Eur J Microbiol Immunol. 2019;9:29–31.
20. Chen L, Li X, Zou T, Wang T, Cui X, Chen Y, et al. Ultrasensitive detection of *H. pylori* in human feces based on immunomagnetic bead capture and fluorescent quantum dots. Analyst. 2019;144:4086–92.
21. Shimoyama T. Stool antigen tests for the management of *Helicobacter pylori* infection. World J Gastroenterol. 2013;19:8188–91.
22. Shimizu T, Fujii T, Haruna H, Shoji H, Kudo T, Yamashiro Y. Effects of stool dilution on the faecal *Helicobacter pylori* antigen test. J Paediatr Child Health. 2003;39:286–8.
23. Gisbert JP, Trapero M, Calvet X, Mendoza J, Quesada M, Güell M, et al. Evaluation of three different tests for the detection of stool antigens to diagnose *Helicobacter pylori* infection in patients with upper gastrointestinal bleeding. Aliment Pharmacol Ther. 2004;19:923–9.

13

Specific Conditions: Children

Hye Ran Yang

Abstract

Helicobacter pylori (*H. pylori*) infection is usually acquired during childhood, causing various diseases even in children. When applying diagnostic tests to children suspected of *H. pylori* infection, there are some differences between children and adults, although endoscopy with biopsies is also recommended for the initial diagnosis of *H. pylori* infection in children. On endoscopy, nodular gastritis is a pathognomonic gross finding of childhood *H. pylori* infection. On histopathology of gastric tissues, infiltration of lymphocytes and plasma cells and formation of mucosa-associated lymphoid tissue are the prominent features in children. Noninvasive diagnostic methods are also available and well validated in children. Both the ^{13}C-urea breath test (UBT) and the 2-step monoclonal *H. pylori* stool antigen test are reliable in identifying *H. pylori* status before and after eradication therapy. However, when applying UBT to children younger than 6 years, test results should be interpreted cautiously because they tend to have high false positives. Serologic tests or urine tests are not recommended in children because of low diagnostic accuracy. As for treatment of *H. pylori* infection in children, recent updates suggest that anti-microbial susceptibility should be considered for infecting *H. pylori* strains before starting first-line treatment, and then tailored eradication therapy should be provided to pediatric patients.

Keywords

Child · Diagnosis · Endoscopy · *Helicobacter pylori* · Noninvasive · Treatment

13.1 Introduction

Helicobacter pylori (*H. pylori*) infection is a common infectious disease of the gastrointestinal (GI) tract in children [1]. Although its prevalence is decreasing in many countries [1, 2], *H. pylori* infection is still prevalent in children and adolescents worldwide [3]. According to a recent meta-analysis reported in 2022, the overall global prevalence of *H. pylori* infection in children was 32.3% (95% CI, 27.3–37.8), revealing significantly higher prevalence in low- to middle-income countries and in older children [3].

H. pylori infection is mainly acquired during early childhood [4], and most of the infected children are asymptomatic initially. However, it can cause various GI diseases such as gastritis, gastric or duodenal ulcer, and mucosa-associated lymphoid tissue (MALT) lymphoma, even in

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children, as well as extraintestinal diseases such as growth retardation and iron deficiency anemia [5].

When applying diagnostic tests to children suspected of *H. pylori* infection, it is obvious that there are some differences between children and adults. Endoscopy with biopsies is basically recommended for the initial diagnosis of *H. pylori* infection even in children. However, endoscopic investigation is somewhat difficult to apply to young children because of its invasiveness. Thus, noninvasive diagnostic tests are preferred in the pediatric population in clinical practice. With the development of equipment and techniques, endoscopy is now available for all ages. Moreover, a variety of noninvasive methods, including the urea breath test (UBT) and the *H. pylori* stool antigen (HpSA) test, have been validated to diagnose *H. pylori* infection in children. Hence, proper diagnosis and treatment strategies for *H. pylori* infection are required in children.

In this chapter, updates on the diagnostic and therapeutic approach to *H. pylori* infection in children will be systematically reviewed.

13.2 Endoscopic Diagnosis of *H. pylori* Infection in Children

13.2.1 Application of Endoscopy with Biopsy in Children

According to the updated guidelines from ESPGHAN (European Society for Paediatric Gastroenterology, Hepatology, and Nutrition) and NASPGHAN (North American Society for Paediatric Gastroenterology, Hepatology, and Nutrition) for *H. pylori* infection in children published in 2017, diagnostic testing such as endoscopy should only be used to investigate the underlying cause of the GI symptom itself and not merely to detect the presence of *H. pylori* infection [5]. Upper endoscopy and biopsy-based testing for *H. pylori* should be considered in a child who is suffering from iron deficiency anemia refractory to long-term iron supplementation therapy without any other causes of anemia; noninvasive diagnostic testing is suggested in a child with chronic immune thrombocytopenic purpura [5]. The updated guidelines from ESPGHAN/NASPGHAN in 2017 recommended that testing for *H. pylori* be done in children with gastric- or duodenal ulcers [5].

According to a recently updated guidelines for *H. pylori* infection in children, endoscopy is not recommended in an individual with functional abdominal pain or as an initial investigation in a child with iron deficiency anemia or short stature [5].

During upper endoscopy, additional biopsies for rapid urease test and culture should only be taken if treatment may be offered when infection is confirmed [5]. Basic investigations for the initial diagnosis of *H. pylori* infection in children are rapid urease test and gastric tissue staining or culture for *H. pylori* using gastric tissues obtained during upper GI endoscopy, just as in adults [6]. Either when histopathology plus at least 1 other biopsy-based test is positive or when culture is positive, it is reasonable to confirm *H. pylori* infection in children, according to the updated guidelines from ESPGHAN and NASPGHAN [5]. Before testing for *H. pylori*, proton pump inhibitor (PPI) should be stopped at least for 2 weeks and antibiotics for 4 weeks [5].

As for histological diagnosis of *H. pylori* infection on endoscopy, at least six gastric biopsies be obtained from the antrum and the body [5]. These tissues are stained with hematoxylin–eosin to evaluate the severity of acute and chronic inflammation, atrophy, and intestinal metaplasia, as per the updated Sydney classification for the histopathological findings of gastritis [7]. Special staining including the Warthin–Starry silver stain, Giemsa stain, or modified Genta stain, and additional immunohistochemistry may aid in the diagnosis of *H. pylori* infection [7].

If the test results of the rapid urease test and histology do not match at all, additional noninvasive diagnostic tests such as the UBT or the HpSA test may be helpful [5].

13.2.2 Endoscopic Findings in *H. pylori*-Infected Children

When mucosal nodularity in the gastric antrum or ulceration in the duodenal bulb is noted on upper GI endoscopy, *H. pylori* infection can be suspected in children [5].

Nodular gastritis or follicular gastritis has a gross appearance of numerous gooseflesh-like small nodules on the gastric mucosa, which is a common endoscopic finding in children with *H. pylori* infection [8, 9]. During the chronic course of *H. pylori* infection, antral nodularity (gastric lymphoid hyperplasia) was observed in 44–67% of *H. pylori*-infected children, in contrast to 0.19% of *H. pylori*-infected adults [9, 10]. Additionally, when nodular gastritis is observed on endoscopy, *H. pylori* can be present in approximately 90% of pediatric patients. Thus, endoscopic manifestations of gastric mucosa in children with *H. pylori* infection are somewhat different from those of adults, and nodular gastritis can be a pathognomonic endoscopic finding of childhood *H. pylori* infection with relatively high specificity but low sensitivity [7]. Since *H. pylori* infection is acquired during childhood, it can cause chronic gastritis, mainly in the form of nodular gastritis.

Gastric cancer or MALT lymphoma is extremely rare during childhood. However, as reported in a previous study in adults, histopathological findings of antral nodularity caused by *H. pylori* infection are similar to those of early-stage gastric MALT lymphoma [11]. Moreover, a recent study in children also reported that severe nodular gastritis in the antrum on endoscopy can indicate gastric MALT on histology even in *H. pylori*-infected children [8]. Because the degree of nodularity on endoscopy may correlate with the grades of gastric MALT, histopathological evaluation for gastric MALT may be recommendable in cases of *H. pylori*-infected children with severe nodular gastritis on endoscopy.

13.2.3 Histopathologic Findings in *H. pylori*-Infected Children

For the diagnosis of *H. pylori* infection in children on the basis of histopathological investigation, along with the rapid urease test or culture for *H. pylori*, the updated Sydney classification should be applied to determine the presence and the severity of acute and chronic inflammation, atrophy, and intestinal metaplasia [5, 7].

Nodular gastritis, a pathognomonic endoscopic finding in *H. pylori*-infected children, indicates a high grade of *H. pylori* colonization and chronic active gastritis with lymphoid follicles with germinal center or MALT, exaggerated by inflammatory reaction caused by *H. pylori* infection [12, 13], and its severity may deteriorate as time passes [14]. In previous studies, antral nodularity in children was correlated with higher histological scores on the gastric mucosa, indicating higher severity of gastritis, and was also associated with a higher density of *H. pylori* and *cagA*-positive strains [15, 16].

Histological findings of *H. pylori*-associated chronic gastritis in children are somewhat different from those in adults because lymphocyte or plasma cell infiltration is more prominent, and neutrophil infiltration is less prominent in children [17, 18].

Atrophy or intestinal metaplasia of gastric mucosa is a rare pathologic finding in children [19]. However, one study reported a relatively high prevalence of gastric atrophy and low-grade intestinal metaplasia in *H. pylori*-infected children compared to uninfected children [20].

13.3 Noninvasive Diagnosis of *H. pylori* Infection in Children

Because endoscopic diagnosis of *H. pylori* infection is relatively costly and invasive to apply to children, there are some limitations, especially in posttreatment settings. Therefore, noninvasive diagnostic tests with high diagnostic accuracy are preferred in childhood to detect *H. pylori* infection. There are several noninvasive diagnostic tests available in children such as UBT, HpSA test, and *H. pylori* antibody tests on serum, urine, or saliva [5, 21].

13.3.1 Urea Breath Test in Children

UBT is one of the most widely used noninvasive diagnostic methods in children. It is very safe, economical, and easy to perform. In addition, UBT is a very accurate noninvasive test to identify *H. pylori* infection even in children and adolescents.

Previously noted diagnostic accuracy of the UBT is high enough to diagnose *H. pylori* infection, with sensitivity and specificity of about 95%, even in children [21, 22]. A recent meta-analysis on ^{13}C-UBT for the diagnosis of *H. pylori* infection in children revealed that UBT has excellent diagnostic accuracy in all ages with a sensitivity of 95.7%, a specificity of 95.7%, a positive likelihood ratio (LR+) of 42.6, and a negative likelihood ratio (LR-) of 0.06 [22]. In children aged 6 years or more, high accuracy was observed, with a sensitivity of 96.6%, a specificity of 97.7%, an LR+ of 42.6, and an LR− of 0.04.

Meanwhile, children less than 6 years of age have relatively high false positives and low specificity compared to children older than 6 years of age and adults [23, 24]. According to a previous study, false positives were about 8.3% in children younger than 6 years of age compared to 0.85% in children older than 6 years [24]. A recent meta-analysis on ^{13}C-UBT in children also confirmed that UBT has lower accuracy, especially lower specificity, in children aged 6 years or less, with a sensitivity of 95%, a specificity of 93.5%, an LR+ of 11.7, and an LR− of 0.12 [22]. Hence, the clinical application of UBT can be somewhat limited in this age group.

There are several hypotheses to explain high false-positive results in young children aged 6 years or less. The first hypothesis is oral urease-producing microorganisms such as *Streptococcus salivarius*, *Proteus mirabilis*, and *Klebsiella pneumonia* [25]. These bacteria colonize the oral cavity of young children, and young children tend to hold the urea in the mouth [25]. This hypothesis was supported by another study that administered urea through a nasogastric tube or a gastrostomy site to avoid the degradation of urea by oral flora [26]. Thus, young children are encouraged to rinse their mouths with clean water after ingesting urea to improve the accuracy of UBT [5].

The second hypothesis is endogenous CO_2 production that differs according to age, sex, and body weight and height [27]. Since UBT measures the isotopic ratio of $^{13}CO_2/^{12}CO_2$, the ingestion of an identical amount of ^{13}C-urea regardless of age, body weight, and height may increase the isotopic ratio of $^{13}CO_2/^{12}CO_2$ in a small child. In previous studies, the application of urea hydrolysis rate adjusted for age, weight, and height, instead of the conventional cutoffs using a delta over baseline, revealed better outcomes [27, 28]. Therefore, although the ^{13}C-UBT test is less accurate in assessing *H. pylori* infection status in children younger than 6 years, this limitation may be improved by adjusting cutoff values, pretest meals, or the dosage of administered urea [22]. In a previous study, applying a cutoff value of 7.0‰ to children aged 6 years or less, instead of the conventional cutoff value of 2.4–4.0‰, yielded better accuracy [24].

The sensitivity and specificity of UBT reach almost 100% after eradication therapy in children. According to the recent guideline from ESPGHAN and NASPGHAN for *H. pylori* infection in children, ^{13}C-UBT is recommended as the most reliable noninvasive method that can replace invasive endoscopy to determine whether *H. pylori* has been eradicated [5, 21].

13.3.2 *H. pylori* Stool Antigen Test in Children

The HpSA test using enzyme-linked immunosorbent assay (ELISA) with polyclonal or monoclonal antibodies is an effective noninvasive test with high diagnostic accuracy to identify *H. pylori* infection in children of all ages [29–31]. According to a recent meta-analysis study on HpSA testing in children, the diagnostic accuracy of ELISA monoclonal antibody revealed the best performance with a pooled sensitivity of 97%, a specificity of 97%, an LR+ of 29.9, and an LR− of 0.03 [30]. On the other hand, the HpSA test using ELISA polyclonal antibody had relatively lower accuracy with a sensitivity of 92%, a specificity of 93%, an LR+ of 16.2, and an LR− of 0.09, with high heterogeneity [30]. In another meta-analysis in children, the sensitivity and the specificity of the monoclonal HpSA test were 96.2% and 94.7%, respectively, whereas those of the polyclonal HpSA test were 88.0% and 93.0%, respectively, also suggesting better accuracy with monoclonal techniques [31]. According to the updated guidelines from ESPGHAN and NASPGHAN for *H. pylori* infection in children, the accuracy of a 2-step monoclonal HpSA test is comparable to that of the ^{13}C-UBT and more convenient in children aged 6 years or less because its accuracy is not influenced by age, and it is easy to perform, simply requiring collection of stool samples [5].

The ELISA HpSA test is also a useful noninvasive method after eradication therapy [32]. The updated guidelines from ESPGHAN and NASPGHAN for *H. pylori* infection in children recommended a 2-step monoclonal HpSA test as a reliable method to determine *H. pylori* status after eradication therapy [5].

A one-step rapid stool antigen test using immunochromatography is also available in children, albeit with relatively low accuracy compared to the HpSA ELISA test [33–35]. Diagnostic accuracy of the one-step HpSA test using monoclonal antibody revealed a sensitivity of 88%, a specificity of 93%, an LR+ of 10.6, and an LR− of 0.11, according to a meta-analysis study on pediatric population [30]. Another recent meta-analysis showed a sensitivity of 88.1% and a specificity of 94.2% for the one-step rapid monoclonal HpSA test in children [31].

After eradication therapy, the weighted mean sensitivity and specificity were 80.9% (71.4–88.2%) and 97.2% (94.8–98.7%), respectively, with an LR+ of 19.71 and an LR− of 0.171 in children. Meanwhile, weighted mean sensitivity and specificity were 92.6% (91.4–93.6%) and 93.8% (93.0–94.6%), respectively, with an LR+ of 16.33 and an LR− of 0.078 before therapy [31]. After the eradication therapy of *H. pylori* infection in children, the diagnostic accuracy of the conventional HpSA test using ELISA monoclonal antibody was reported to be relatively higher than the rapid HpSA test using monoclonal antibody in children [33–35].

13.3.3 *H. pylori* Antibody Tests

Many serologic tests to detect IgA and IgG antibodies against *H. pylori* are commercially available and have been applied in children for epidemiological studies and in clinical practice [21]. The serologic test may be useful in screening *H. pylori* infection, but its application has been limited in young children owing to low sensitivity. According to a meta-analysis on antibody-based diagnostic tests for *H. pylori* infection in children reported in 2008, serologic tests using ELISA IgG antibody had a low sensitivity of 79.2% (95% confidence interval [CI], 77.3–81.0) and a high specificity of 92.4% (95% CI, 91.6–93.3), with heterogeneity [36]. Antibody titers are reported to be relatively lower in young children because of the short infection period and immature immune response to *H. pylori* [37, 38]. On the other hand, a meta-analysis on children reported that antibody-based test using Western blots had relatively good performance even in children, with a sensitivity of 91.3% (95% CI, 88.9–93.3), a specificity of 89% (95% CI, 85.7–91.9), an LR+ of 8.2 (95% CI, 5.1–13.3), and an LR− of 0.06 (95% CI, 0.02–0.16) [39].

The detection of serum *H. pylori* IgG antibody is not appropriate for monitoring *H. pylori* status after treatment because serum IgG antibody titers can remain elevated for a long time even after *H. pylori* is eradicated [39].

H. pylori antibodies are also detectable in urine and saliva, as well as in blood, but these antibody tests are not recommended because of their low accuracy in the pediatric population [21]. Therefore, the guidelines from ESPGHAN and NASPGHAN for *H. pylori* infection in children stated that antibody tests against *H. pylori* in serum, urine, or saliva are not recommended because these are not reliable for use in children [5].

13.4 Treatment of *H. pylori* Infection in Children

According to the updated guidelines from ESPGHAN/NASPGHAN for *H. pylori* infection in children published in 2017, a “test and treat” strategy for *H. pylori* infection is not recommended in pediatric patients [5]. If *H. pylori* infection is incidentally detected at endoscopy, treatment may be considered after careful discussion of the benefits and risks of *H. pylori* eradication treatment with the patient and parents [5]. In children with gastric and duodenal ulcers at upper GI endoscopy, testing for *H. pylori* is recommended, and treatment should be advised if *H. pylori* infection is identified [5]. Based on the updated guidelines from ESPGHAN/NASPGHAN, the effectiveness of first-line treatment should be evaluated in each country and region, and antibiotic susceptibility should be obtained for infecting *H. pylori* strains before starting treatment, and then tailored eradication therapy should be provided accordingly [5]. It is important for physicians to explain to the patient/family the importance of adherence to medication for successful eradication [5].

Recommended options for first-line therapy for *H. pylori* infection in children may vary according to *H. pylori* anti-microbial susceptibility; (1) PPI–amoxicillin–clarithromycin triple therapy with a standard dose for 14 days if susceptible to both clarithromycin and metronidazole; (2) PPI–amoxicillin–metronidazole triple therapy for 14 days if resistant to clarithromycin and susceptible to metronidazole; (3) PPI–amoxicillin–clarithromycin triple therapy for 14 days if resistant to metronidazole and susceptible to clarithromycin; (4) PPI–amoxicillin (high dose)–metronidazole or bismuth-based triple therapy for 14 days if resistant to both clarithromycin and metronidazole; and (5) high-dose PPI–amoxicillin–metronidazole or bismuth-based triple therapy or PPI–amoxicillin–metronidazole–clarithromycin quadruple therapy for 14 days if antibiotic susceptibility is unknown [5].

According to the data from the EuroPedHp registry of the *Helicobacter pylori* Special Interest Group of ESPGHAN in 2022, the majority of pediatric patients diagnosed with *H. pylori* infection were treated with tailored triple therapy of PPI plus amoxicillin combining with either clarithromycin or metronidazole for 14 days [40]. In treatment-naïve patients, eradication was obtained in 90% and 93% of children with good compliance. Treatment failure was associated with clarithromycin resistance (OR 2.47; 95% CI, 1.10–5.53), metronidazole resistance (OR 3.44; 95% CI, 1.47–8.08), and low compliance (OR 5.89; 95% CI, 2.49–13.95) [40]. In this European study, resistance to metronidazole was noted in 21%, clarithromycin in 28.8%, and both in 7.1% [40].

According to the updated 2017 guidelines from ESPGHAN/NASPGHAN, the outcome of eradication therapy against *H. pylori* infection should be assessed at least 4 weeks after the completion of therapy using the ^{13}C-UBT or 2-step monoclonal HpSA test [5]. When eradication fails, rescue therapy against *H. pylori* infection should be individualized considering age, antibiotic susceptibility, and available drugs [5].

13.5 Conclusion

Diagnosis of *H. pylori* infection in children has some unique features and different diagnostic accuracy compared to that of adults. Antral nodularity on upper endoscopy may be a pathognomonic finding of *H. pylori* infection in childhood. Histopathological findings of gastric mucosa show predominant lymphocytes and plasma cell infiltration, forming gastric MALT. Among non-invasive diagnostic tests, UBT and HpSA tests are both preferred in childhood as they have good accuracy on testing before and after eradication therapy. UBT has high false positives in children aged 6 years or less, and thus, the HpSA test with ELISA monoclonal antibody may be more beneficial in young children. Finally, antibody-based tests using the ELISA IgG antibody technique are not recommended in children because of low sensitivity.

Therefore, when determining the diagnostic test to identify *H. pyloris* status in children, whether it is invasive or noninvasive, special consideration regarding these unique aspects is required in children.

References

1. Mégraud F. Epidemiology of *Helicobacter pylori* infection. Gastroenterol Clin N Am. 1993;22:73–88.
2. Miyamoto R, Okuda M, Lin Y, Murotani K, Okumura A, Kikuchi S. Rapidly decreasing prevalence of *Helicobacter pylori* among Japanese children and adolescents. J Infect Chemother. 2019;25:526–30.
3. Yuan C, Adeloye D, Luk TT, Huang L, He Y, Xu Y, et al. The global prevalence of and factors associated with *Helicobacter pylori* infection in children: a systematic review and meta-analysis. Lancet Child Adolesc Health. 2022;6:185–94.
4. Rothenbacher D, Inceoglu J, Bode G, Brenner H. Acquisition of *Helicobacter pylori* infection in a high-risk population occurs within the first 2 year of life. J Pediatr. 2000;136:744–8.
5. Jones NL, Koletzko S, Goodman K, Bontems P, Cadranel S, Casswall T, et al. Joint ESPGHAN/NASPGHAN guidelines for the management of *Helicobacter pylori* in children and adolescents (update 2016). J Pediatr Gastroenterol Nutr. 2017;64:991–1003.
6. Thijs JC, van Zwet AA, Thijs WJ, Oey HB, Karrenbeld A, Stellaard F, et al. Diagnostic test for *Helicobacter pylori*: a prospective evaluation of their accuracy, without selecting a single test as the gold standard. Am J Gastroenterol. 1996;91:2125–9.
7. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney system. International workshop on the histopathology of gastritis, Houston 1994. Am J Surg Pathol. 1996;20:1161–81.
8. Yang HR, Choi HS, Paik JH, Lee HS. Endoscopic and histologic analysis of gastric mucosa-associated lymphoid tissue in children with *Helicobacter pylori* infection. J Pediatr Gastroenterol Nutr. 2013;57:298–304.
9. Prieto G, Polanco I, Larrauri J, Rota L, Lama R, Carrasco S. *Helicobacter pylori* infection in children: clinical, endoscopic, and histologic correlations. J Pediatr Gastroenterol Nutr. 1992;14:420–5.
10. Miyamoto M, Haruma K, Yoshihara M, Hiyama T, Sumioka M, Nishisaka T, et al. Nodular gastritis in adults is caused by *Helicobacter pylori* infection. Dig Dis Sci. 2003;48:968–75.
11. Ma ZQ, Tanizawa T, Nihei Z, Sugihara K, Nakamura K. Follicular gastritis associated with *Helicobacter pylori*. J Med Dent Sci. 2000;47:39–47.
12. Bahu' Mda G, da Silveira TR, Maguilnick I, Ulbrich-Kulczynski J. Endoscopic nodular gastritis: an endoscopic indicator of high-grade bacterial colonization and severe gastritis in children with *Helicobacter pylori*. J Pediatr Gastroenterol Nutr. 2003;36:217–22.
13. Riddell HR. Pathobiology of *Helicobacter pylori* infection in children. Can J Gastroenterol. 1999;13:599–603.
14. Ganga-Zandzou PS, Michaud L, Vincent P, Husson MO, Wizla-Derambure N, Delassalle EM, et al. Natural outcome of *Helicobacter pylori* infection in asymptomatic children: two-year follow-up study. Pediatrics. 1999;104(2 Pt 1):216–21.
15. Uhlig HH, Tannapfel A, Mössner J, Jedwilayties S, Deutscher J, Müller DM, et al. Histopathological parameters of *Helicobacter pylori*-associated gastritis in children and adolescents: comparison with findings in adults. Scand J Gastroenterol. 2003;38:701–6.
16. Gallo N, Zambon CF, Navaglia F, Basso D, Guariso G, Grazia Piva M, et al. *Helicobacter pylori* infection in children and adults: a single pathogen but a different pathology. Helicobacter. 2003;8:21–8.
17. Whitney AE, Guarner J, Hutwagner L, Gold BD. *Helicobacter pylori* gastritis in children and adults: comparative histopathologic study. Ann Diagn Pathol. 2000;4:279–85.
18. Queiroz DM, Rocha GA, Mendes EN, Carvalho AS, Barbosa AJ, Oliveira CA, et al. Differences in distribution and severity of *Helicobacter pylori* gastritis in children and adults with duodenal ulcer disease. J Pediatr Gastroenteriol Nutr. 1991;12:178–81.
19. Meining A, Behrens R, Lehn N, Bayerdörffer E, Stolte M. Different expression of *Helicobacter pylori* gastritis in children: evidence for a specific pediatric disease? Helicobacter. 1996;1:92–7.
20. Kim KM, Oh YL, Ko JS, Choe YH, Seo JK. Histopathology and expression of Ki-67 and cyclooxygenase-2 in childhood *Helicobacter pylori* gastritis. J Gastroenterol. 2004;39:231–7.
21. Guarner J, Kalach N, Elitsur Y, Koletzko S. *Helicobacter pylori* diagnostic tests in children: review of the literature from 1999 to 2009. Eur J Pediatr. 2010;169:15–25.
22. Leal YA, Flores LL, Fuentes-Pananá EM, Cedillo-Rivera R, Torres J. 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection in children: a systematic review and meta-analysis. Helicobacter. 2011;16:327–37.
23. Kindermann A, Demmelmair H, Koletzko B, Krauss-Etschmann S, Wiebecke B, Koletzko S. Influence of age on ^{13}C-urea breath test results in children. J Pediatr Gastroenterol Nutr. 2000;30:85–91.
24. Yang HR, Seo JK. Diagnostic accuracy of the ^{13}C-urea breath test in children: adjustment of the cut-off value according to age. J Gastroenterol Hepatol. 2005;20:264–9.
25. Jones NL, Sherman PM. Approaching *Helicobacter pylori* infection in children: level I evidence at last and a word of caution. J Pediatr. 2001;139:622–3.
26. Peng NJ, Lai KH, Liu RS, Lee SC, Tsay DG, Lo CC, et al. Clinical significance of oral urease in diagnosis of *Helicobacter pylori* infection by ^{13}Curea breath test. Dis Dis Sci. 2001;46:1772–8.
27. Klein PD, Malaty HM, Czinn SJ, Emmons SC, Martin RF, Graham DY. Normalizing results of ^{13}C-urea

breath testing for CO_2 production rates in children. J Pediatr Gastroenterol Nutr. 1999;29:297–301.

28. Yang HR, Ko JS, Seo JK. Does the diagnostic accuracy of the ^{13}C-urea breath test vary with age even after the application of urea hydrolysis rate? Helicobacter. 2008;13:239–44.
29. Konstantopoulos N, Rüssmann H, Tasch C, Sauerwald T, Demmelmair H, Autenrieth I, et al. Evaluation of the *Helicobacter pylori stool antige*n test (HpSA) for detection of *Helicobacter pylori* infection in children. Am J Gastroenterol. 2001;96:677–83.
30. Leal YA, Cedillo-Rivera R, Simón JA, Velázquez JR, Flores LL, Torres J. Utility of stool sample-based tests for the diagnosis of *Helicobacter pylori* infection in children. J Pediatr Gastroenterol Nutr. 2011;52:718–28.
31. Zhou X, Su J, Xu G, Zhang G. Accuracy of stool antigen test for the diagnosis of *Helicobacter pylori* infection in children: a meta-analysis. Clin Res Hepatol Gastroenterol. 2014;38:629–38.
32. Gosciniak G, Przondo-Mordarska A, Iwanczak B, Blitek A. *Helicobacter pylori* antigens in stool specimens of gastritis children before and after treatment. J Pediatr Gastroenterol Nutr. 2003;36:376–80.
33. Yang HR, Seo JK. *Helicobacter pylori* stool antigen (HpSA) tests in children before and after eradication therapy: comparison of rapid immunochromatographic assay and HpSA ELISA. Dig Dis Sci. 2008;53:2053–8.
34. Schwarzer A, Lottspeich C, Rüssmann H, Ossiander G, Koletzko S. Evaluation of a novel rapid one-step monoclonal chromatographic immunoassay for detection of *Helicobacter pylori* in stool from children. Eur J Clin Microbiol Infect Dis. 2007;26:475–80.
35. Antos D, Crone J, Konstantopoulos N, Koletzko S. Evaluation of a novel rapid one-step immunochromatographic assay for detection of monoclonal *Helicobacter pylori* antigen in stool samples from children. J Clin Microbiol. 2005;43:2598–601.
36. Leal YA, Flores LL, García-Cortés LB, Cedillo-Rivera R, Torres J. Antibody-based detection tests for the diagnosis of *Helicobacter pylori* infection in children: a meta-analysis. PLoS One. 2008;3:e3751.
37. Czinn SJ. Serodiagnosis of *Helicobacter pylori* in pediatric patients. J Pediatr Gastroenterol Nutr. 1999;28:132–4.
38. Graham DY, Erans DJ Jr, Peacock J, Baker JJ, Scarier WH. Comparison of rapid serological tests (flexure HP and quick Vue) with conventional ELISA for detection of *Helicobacter pylori* infection. Am J Gastroenterol. 1996;91:942–8.
39. Veenendaal RA, Pena AS, Meijer JL, Endtz HP, van der Est MM, van Duijn W, et al. Long term serological surveillance after treatment of *Helicobacter pylori* infection. Gut. 1991;32:1291–4.
40. Le Thi TG, Werkstetter K, Kotilea K, Bontems P, Cabral J, Cilleruelo et al. Management of *Helicobacter pylori* infection in paediatric patients in Europe: results from the EuroPedHp Registry. Infection. 2023;51:921–34.

14

Specific Conditions: Diagnosis of *H. pylori* Infection in Case of Upper Gastrointestinal Bleeding

Yoon Jin Choi

Abstract

The main causes of upper gastrointestinal (GI) bleeding are peptic ulcer diseases such as benign gastric ulcer (BGU) or duodenal ulcer (DU). *Helicobacter pylori* (*H. pylori*) infection was detected up to 70% in patients with BGU and 90% in patients with DU. Since many studies demonstrated the benefit of *H. pylori* eradication in decreasing peptic ulcer recurrences as well as in bleeding cases, it is very important to diagnose the infection accurately among those with peptic ulcer diseases. There are several tests for the detection of *H. pylori* with variable levels of sensitivity and specificity, and upper GI bleeding would reduce sensitivity. In the case of upper GI bleeding, the ^{13}C-urea breath test is more sensitive than tests with biopsied mucosa such as rapid urease test, histological test, or culture study.

Keywords

Bleeding · *Helicobacter pylori* · Rapid urease test · Urea breath test

14.1 Introduction

Upper gastrointestinal (GI) bleeding is the most serious complication of peptic ulcer diseases, which can lead to mortality. The well-known risk factors for peptic ulcer diseases are *Helicobacter pylori* (*H. pylori*) infection and the administration of non-steroidal anti-inflammatory drugs (NSAID). When the subjects with upper GI bleeding or NSAID users were excluded, *H. pylori* infection was detected in up to 70% of patients with benign gastric ulcer (BGU) and 90% in patients with duodenal ulcer (DU) [1]. Since *H. pylori* infection is a single predictive factor for re-bleeding in patients with DU [2], and eradication of the organism can reduce the risk of re-bleeding from 23.7% to 4.5% [3, 4], international guidelines have recommended the tests for diagnosis of *H. pylori* infection in case of upper GI bleeding with peptic ulcer disease [5].

It has been reported that patients with upper GI bleeding showed lower *H. pylori* positivity than those without bleeding [6, 7]. Although this result suggested that peptic ulcer disease with the bleeding might have a different pathophysiology rather than *H. pylori*, it is more reasonable that the bleeding may decrease in sensitivity of detection of *H. pylori,* leading to the underestimation of its importance. However, the international guidelines on peptic ulcer bleeding do not provide clear guidance for optimal testing methods

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in the acute bleeding setting. An optimal strategy for the available *H. pylori* tests is needed.

Therefore, the sensitivity and specificity of various *H. pylori* tests in case of upper GI bleeding were reviewed.

14.2 Accuracy of Diagnostic Methods for *H. Pylori* in Upper Gastrointestinal Bleeding

14.2.1 Invasive Tests

14.2.1.1 Rapid Urease Test (RUT)

Since most patients with upper GI bleeding undergo upper GI endoscopy, rapid urease test (RUT) is the most common examination in this setting. It has been reported that RUT using gastric antral mucosa showed a high probability of false negativity in Hong Kong [8] in accordance with several studies [9–11]. Our laboratory also reported a consistent result that the sensitivity of RUT was lower than histology in patients with peptic ulcer bleeding [12].

The precise reason for this is unclear, but there are several mechanisms proposed for the false-negative result of RUT during peptic ulcer bleeding. The bactericidal effect of serum induces a transient decrease in bacterial density. The presence of anti-*H. pylori* antibodies inhibits urease production, while serum enzymes or electrolytes suppress urease activity. Various buffering systems, such as albumin, bicarbonate, and phosphate, interfere with the pH level of the RUT reagent. Concomitant administration of proton pump inhibitors (PPIs) has also been suggested [13]. In one in vitro study [14], a false-negative RUT result was caused by the buffering effects of serum albumin on the pH indicator but not on urease activity. Another in vitro study concluded that a large amount of gastric lavage before endoscopy could result in a false-negative RUT result [15]. However, Tu TC et al. [16] found no influence on the likelihood of a false-negative result if the gastric antral biopsy specimen was cleansed by normal saline before inoculating the wells for the CLOtest. Similarly, another study concluded that an artificial blood-soaked antral specimen did not influence the results of two RUTs [17]. The bactericidal effect of human plasma [18, 19] and the reduction in bacterial load by PPIs [20] have been demonstrated. Another possible consideration is that the patchy distribution of *H. pylori*, thus using samples from the gastric antrum only, may be inadequate. Blood in the stomach could induce *H. pylori* migration to the gastric body or fundus and the decrease bacterial density of the antrum. Fewer amounts of specimens could be obtained during emergent endoscopy procedures are other explanations of false-negative results of RUT in patients with upper GI bleeding [13].

Simultaneous gastric antral and body specimens or multiple biopsies have been found to produce more positive RUTs [21, 22]. Although there is a consensus on how many biopsies are appropriate for this situation, it was reported that RUT using four antral biopsies was more sensitive than that with one piece of a biopsy sample from gastric antrum (61% vs. 74%); the former yielded the sensitivity as the same as RUT using one piece of a biopsy sample from the gastric body (73% vs. 74%) [23].

Most authors concluded that the RUT cannot be the only diagnostic test in such circumstances [12, 24]. If the initial diagnostic test is negative, a delayed test 4–8 weeks later can have up to an 80% positive rate in previously negative patients [13, 25].

14.2.1.2 Histology

Low sensitivity with histologic methods in patients with bleeding peptic ulcers was reported, which is consistent with RUT sensitivity [9]. However, other studies have reported that histology is more sensitive than RUT [9, 12, 26, 27]. Patchy distribution of bacterial density, staining methods, and pathologist's interpretations also influence the results [13]. The sensitivity of histology also relies on the experience of the endoscopist to take the biopsy from the appropriate site. Therefore, combination tests should be performed to achieve a more precise diagnosis [13].

14.2.1.3 Culture

Culturing *H. pylori* gives additional information such as the susceptibility to certain antibiotics but produced a low yield of sensitivity, 70–90% [28]. The reasons for the relatively high false negativity include the inadequate amount of obtained tissue, time to the inoculation on the culture medium, conditions of the broth, and the micro-anaerobic nature of *H. pylori* [29]. In particular, the author reported that culturing *H. pylori* in patients with bleeding peptic ulcers showed a decrease in sensitivity [12]. Therefore, the culture study should not be considered as the first diagnostic test for *H. pylori* in the upper GI setting due to the time-consuming nature of the process and the lack of time to perform the procedure during endoscopy. However, this method can be used when the patients have resistance to anti-*H. pylori* therapy together with other diagnostic methods.

14.2.1.4 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) using either gastric or buccal mucosa has been used as an invasive test to diagnose *H. pylori* infection [30]. In one study, this test was less sensitive in patients with bleeding peptic ulcers than in those with non-bleeding peptic ulcers and chronic gastritis [31]. However, another study reported that PCR had higher sensitivity than other biopsy-based tests and similar sensitivity to noninvasive tests [32]. It has been also reported that real-time PCR can improve *H. pylori* detection rate in a histology-negative, formalin-fixed, and paraffin-embedded tissue [33]. Since the newly developed quantitative real-time PCR is around ten times more sensitive than the conventional PCR method [34], real-time PCR in the diagnosis of *H. pylori* infection in patients with upper GI bleeding could be useful diagnostic methods with an increase in sensitivity and additional gain of bacterial load [35]. However, further study about the cutoff value, which can distinguish the real infection from asymptomatic colonization, is warranted.

14.2.2 Noninvasive Tests

14.2.2.1 Urea Breath Test

Many studies have confirmed that the ^{13}C-urea breath test (UBT) can accurately diagnose *H. pylori* infection in patients with upper GI bleeding [16, 26, 27, 36]. The sensitivity of this study is not affected by blood in the stomach and is higher than those of biopsy-based methods and other noninvasive tests [37]. Nonetheless, since patients should drink a urea-containing solution with a test meal or citric acid, this method is not suitable for bleeding patients. Therefore, most UBTs are done when patients start eating, or the UBT is reserved as a delayed test if the initial invasive methods are negative. However, using low-dose encapsulated ^{13}C-urea has proven to be feasible in fasting patients or even before an endoscopy because it only takes a small amount of water to swallow the pill [38].

14.2.2.2 *H. pylori* Stool Antigen Test

The *H. pylori* stool antigen test is a relatively accurate noninvasive test, which can be performed by enzyme-linked immunosorbent assay (ELISA) with monoclonal or polyclonal antibodies or by immunochromatographic assay with monoclonal antibodies [13]. The sensitivity of this method is reduced by upper GI bleeding when polyclonal ELISA or immunochromatographic stool antigens are used [36, 39, 40]. Several studies reported a high number of false-positive results in patients with upper GI bleeding due to a cross reaction with the blood [40, 41]. Therefore, the *H. pylori* stool antigen diagnostic test is not recommended for use in patients with upper GI.

14.2.2.3 Serology

The author [12] and others [9] have demonstrated that serology is more sensitive than other invasive tests in cases of bleeding peptic ulcer. Although serologic tests can be used as the initial invasive test, it is mostly performed when other tests are negative. However, commercial serological tests must be confirmed by a local laboratory before they are used in an individual hospital [42]. More importantly, if patients have been treated for *H. pylori* infection, serological tests have revealed that serum antibodies may last for up to a year [42]. This fact must not be overlooked when interpreting the results.

14.3 Appropriate Methods for Detection of *H. pylori* in Upper Gastrointestinal Bleeding

Although guidelines recommend diagnostic tests for *H. pylori* in upper GI bleeding, there has been no consensus about which methods should be used in this setting. In spite of the reduced accuracies of *H. pylori* diagnostic tests in patients with bleeding peptic ulcers, RUT is conducted most frequently for patients with upper GI bleeding because of its convenience and rapidity. Multiple biopsies on both the antrum and body for UBT and combination with other tests raise the detection rate of *H. pylori* [23]. How many pieces of biopsy need to be further studied, but this can apply to the patients differently according to the conditions. If only a single biopsy is permitted, the gastric body is more suitable for the high yield [23]. Repeated biopsy in non-bleeding focus or additional ^{13}C-UBT can increase the accuracy of detecting *H. pylori* [43]. On the contrary, stool antigen tests were less accurate, and serology, although not influenced by upper GI bleeding, was not recommended as the first test.

14.4 Conclusion

Although *H. pylori* infection has been decreasing and NSAID users are increasing, *H. pylori* is still an important risk factor for peptic ulcers. NSAID and *H. pylori* infection may have a synergistic effect on upper GI bleeding in patients with peptic ulcer disease [44], but eradication of *H. pylori* can decrease the likelihood of peptic ulcer rebleeding and associated complications. This suggests that a more accurate diagnosis of *H. pylori* is important in patients with upper GI bleeding. Knowledge of the characteristics and limitations of each *H. pylori* test and the selection of appropriate diagnostic methods in patients with peptic ulcer bleeding could be a basis for the management of peptic ulcer patients.

References

1. Kuipers EJ, Thijs JC, Festen HP. The prevalence of *Helicobacter pylori* in peptic ulcer disease. Aliment Pharmacol Ther. 1995;9 (Suppl 2):59–69.
2. Lai KC, Hui WM, Wong WM. Treatment of *Helicobacter pylori* in patients with duodenal ulcer hemorrhage-a long-term randomized, controlled study. Am J Gastroenterol. 2000;95:2225–32.
3. Graham DY, Hepps KS, Ramirez FC, Lew GM, Saeed ZA. Treatment of *Helicobacter pylori* reduces the rate of rebleeding in peptic ulcer disease. Scand J Gastroenterol. 1993;28:939–42.
4. Gisbert JP, Khorrami S, Carballo F, Calvet X, Gene E, Dominguez-Muñoz E. Metaanalysis: *Helicobacter pylori* eradication therapy vs. antisecretory non-eradication therapy for the prevention of recurrent bleeding from peptic ulcer. Aliment Pharmacol Ther. 2004;19:617–29.
5. Barkun A, Bardou M, Marshall JK. Consensus recommendations for managing patients with nonvariceal upper gastrointestinal bleeding. Ann Intern Med. 2003;139:843–57.
6. Vaira D, Menegatti M, Miglioli M. What is the role of *Helicobacter pylori* in complicated ulcer disease? Gastroenterology. 1997;113:S78–84.
7. Gisbert JP, Pajares JM. *Helicobacter pylori* and bleeding peptic ulcer: what is the prevalence of the infection in patients with this complication? Scand J Gastroenterol. 2003;38:2–9.
8. Lai KC, Hui WM, Lam SK. Bleeding ulcers have high false negative rates for antral *Helicobacter pylori* when tested with urease test. Gastroenterology. 1996;110:A167.
9. Tu TC, Lee CL, Wu CH. Comparison of invasive and noninvasive tests for detecting *Helicobacter pylori* infection in bleeding peptic ulcers. Gastrointest Endosc. 1999;49(3 Pt 1):302–6.
10. Lee JM, Breslin NP, Fallon C, O'Morain CA. Rapid urease tests lack sensitivity in *Helicobacter pylori* diagnosis when peptic ulcer disease presents with bleeding. Am J Gastroenterol. 2000;95:1166–70.
11. Schilling D, Demel A, Adamek HE, Nüsse T, Weidmann E, Riemann JF. A negative rapid urease test is unreliable for exclusion of *Helicobacter pylori* infection during acute phase of ulcer bleeding: a prospective case control study. Dig Liv Dis. 2003;35:217–21.
12. Choi YJ, Kim N, Lim J, Jo SY, Shin CM, Lee HS, et al. Accuracy of diagnostic tests for *Helicobacter pylori* in patients with peptic ulcer bleeding. Helicobacter. 2012;17:77–85.
13. Huang TC, Lee CL. Diagnosis, treatment, and outcome in patients with bleeding peptic ulcers and

Helicobacter pylori infections. Biomed Res Int. 2014;2014:658108.

14. Leung WK, Sung JJ, Siu KL, Chan FK, Ling TK, Cheng AF. False-negative biopsy urease test in bleeding ulcers caused by the buffering effects of blood. Am J Gastroenterol. 1998;93:1914–8.
15. Fantry GT, Rosenstein AH, James SP. Confounding factors in the detection of *Helicobacter pylori* infection in patients with upper gastrointestinal bleeding. Am J Gastroenterol. 1999;94:1421–2.
16. Tu TC, Lee CL, Wu CH. False negative CLOtest in bleeding ulcers can't be corrected by cleansing the implanted specimen. Gut. 1999;45 (Suppl 5):A121.
17. Laine L, Sidhom O, Emami S, Estrada R, Cohen H. Effect of blood on rapid urease testing of gastric mucosal biopsy specimens. Gastrointest Endosc. 1998;47:141–3.
18. Houghton J, Ramamoorthy R, Pandya H, Dhirmalani R, Kim KH. Human plasma is directly bacteriocidal against *Helicobacter pylori* in vitro, potentially explaining the decreased detection of *Helicobacter pylori* during acute upper GI bleeding. Gastrointest Endosc. 2002;55:11–6.
19. Gonzalez-Valencia G, Perez-Perez GI, Washburn RG, Blaser MJ. Susceptibility of *Helicobacter pylori* to the bactericidal activity of human serum. Helicobacter. 1996;1:28–33.
20. Graham DY, Opekun AR, Hammoud F, Yamaoka Y, Reddy R, Osato MS, et al. Studies regarding the mechanism of false negative urea breath tests with proton pump inhibitors. Am J Gastroenterol. 2003;98:1005–9.
21. Laine L, Chun D, Stein C, El-Beblawi I, Sharma V, Chandrasoma P. The influence of size or number of biopsies on rapid urease test results: a prospective evaluation. Gastrointest Endosc. 1996;43:49–53.
22. Siddique I, Al-Mekhaizeem K, Alateeqi N, Memon A, Hasan F. Diagnosis of *Helicobacter pylori*: improving the sensitivity of CLOtest by increasing the number of gastric antral biopsies. J Clin Gastroenterol. 2008;42:356–60.
23. Lee TH, Lin CC, Chung CS, Lin CK, Liang CC, Tsai KC. Increasing biopsy number and sampling from gastric body improve the sensitivity of rapid urease test in patients with peptic ulcer bleeding. Dig Dis Sci. 2015;60:454–7.
24. Chan HL, Wu JC, Chan FK, Choi CL, Ching JY, Lee YT, et al. Is non-*Helicobacter pylori*, non-NSAID peptic ulcer a common cause of upper GI bleeding? A prospective study of 977 patients. Gastrointest Endosc. 2001;53:438–42.
25. Güell M, Artigau E, Esteve V, Sánchez-Delgado J, Junquera F, Calvet X. Usefulness of a delayed test for the diagnosis of *Helicobacter pylori* infection in bleeding peptic ulcer. Aliment Pharmacol Ther. 2006;23:53–9.
26. Chung IK, Hong SJ, Kim EJ, Cho J, Kim H, Park S, et al. What is the best method to diagnose *Helicobacter* infection in bleeding peptic ulcers?: A prospective trial. Korean J Intern Med. 2001;16:147–52.
27. Griñó P, Pascual S, Such J, Casellas M, Niveiro M, Andreu J, et al. Comparison of diagnostic methods for *Helicobacter pylori* infection in patients with upper gastrointestinal bleeding. Scand J Gastroenterol. 2001;36:1254–8.
28. Kjøller M, Fischer A, Justesen T. Transport conditions and number of biopsies necessary for culture of *Helicobacter pylori*. Eur J Clin Microbiol Infect Dis. 1991;10:166–7.
29. Cammarota G, Ianiro G, Bibbò S, Di R, Teresa A, Masucci L, Sanguinetti M, et al. Culture-guided treatment approach for *Helicobacter pylori* infection: review of the literature. World J Gastroenterol. 2014;20:5205–11.
30. Lopes AI, Vale FF, Oleastro M. *Helicobacter pylori* infection-recent developments in diagnosis. World J Gastroenterol. 2014;20:9299–313.
31. Lin HJ, Lo WC, Perng CL, Tseng GY, Li AF, Ou YH. Mucosal polymerase chain reaction for diagnosing *Helicobacter pylori* infection in patients with bleeding peptic ulcers. World J Gastroenterol. 2005;11:382–5.
32. Lo CC, Lai KH, Peng NJ, Lo GH, Tseng HH, Lin CK, et al. Polymerase chain reaction: a sensitive method for detecting *Helicobacter pylori* infection in bleeding peptic ulcers. World J Gastroenterol. 2005;11:3909–14.
33. Ramírez-Lázaro MJ, Lario S, Casalots A, Sanfeliu E, Boix L, García-Iglesias P, et al. Real-time PCR improves *Helicobacter pylori* detection in patients with peptic ulcer bleeding. PLoS One. 2011;6:e20009.
34. Shukla SK, Prasad KN, Tripathi A, Ghoshal UC, Krishnani N, Nuzhat H, et al. Quantitation of *Helicobacter pylori ureC* gene and its comparison with different diagnostic techniques and its comparison with different diagnostic techniques and gastric histopathology. J Microbiol Methods. 2011;86:231–7.
35. Saez J, Belda S, Santibáñez M, Rodríguez JC, Sola-Vera J, Galiana A, et al. Real-time PCR for diagnosing *Helicobacter pylori* infection in patients with upper gastrointestinal bleeding: comparison with other classical diagnostic methods. J Clin Microbiol. 2012;50:3233–7.
36. Griño P, Pascual S, Such J, Casellas JA, Niveiro M, Andreu M, et al. Comparison of stool immunoassay with standard methods for detection of *Helicobacter pylori* infection in patients with upper-gastrointestinal bleeding of peptic origin. Eur J Gastroenterol Hepatol. 2003;15:525–9.
37. Gisbert JP, Esteban C, Jimenez I, Moreno-Otero R. ^{13}C-urea breath test during hospitalization for the diagnosis of *Helicobacter pylori* infection in peptic ulcer bleeding. Helicobacter. 2007;12:231–7.
38. Winiarski M, Bielanski W, Plonka M, Dobrzanska M, Kaminska A, Bobrzynski A, et al. The usefulness of capsulated 13C-urea breath test in diagnosis of *Helicobacter pylori* infection in patients with upper gastrointestinal bleeding. J Clin Gastroenterol. 2003;37:34–8.

39. Gisbert JP, Trapero M, Calvet X, Mendoza J, Quesada M, Güell M, et al. Evaluation of three different tests for the detection of stool antigens to diagnose *Helicobacter pylori* infection in patients with upper gastrointestinal bleeding. Aliment Pharmacol Ther. 2004;19:923–9.
40. Lin HJ, Lo WC, Perng CL, Li AF, Tseng GY, Sun IC, et al. *Helicobacter pylori* stool antigen test in patients with bleeding peptic ulcers. Helicobacter. 2004;9:663–8.
41. van Leerdam ME, van der Ende A, ten Kate FJ, Rauws EAJ, Tytgat GNJ. Lack of accuracy of the noninvasive *Helicobacter pylori* stool antigen test in patients with gastroduodenal ulcer bleeding. Am J Gastroenterol. 2003;98:798–801.
42. Wang WM, Chen CY, Jan CM, Chen LT, Perng DS, Lin SR, et al. Long-term follow-up and serological study after triple therapy of *Helicobacter pylori*-associated duodenal ulcer. Am J Gastroenterol. 1994;89:1793–6.
43. Gisbert JP, Abraira V. Accuracy of *Helicobacter pylori* diagnostic tests in patients with bleeding peptic ulcer: a systematic review and meta-analysis. Am J Gastroenterol. 2006;101:848–63.
44. Huang JQ, Sridhar S, Hunt RH. Role of *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. Lancet. 2002;359:14–22.

Diagnosis of *H. pylori* Infection After Gastric Surgery

15

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Abstract

There was a survival improvement after *Helicobacter pylori* (*H. pylori*) eradication in patients treated with surgery not only in the early gastric cancer (GC) but also in the advanced GC. The Maastricht VI/Florescence Consensus strongly recommended *H. pylori* eradication in patients with previous gastric neoplasia treated with endoscopic or subtotal gastric resection to reduce the recurrence of GC. However, as a remnant stomach after gastrectomy has a different anatomic and biological environment, the distribution of *H. pylori* becomes different. These changes in postoperative *H. pylori* infection status have been suggested to be related to bile reflux and dramatic change of acid secretion after the surgery, which appear to inhibit the growth of *H. pylori* in the remnant stomach. In addition, as there are foods in the remained stomach that contain other urease producing bacteria in addition to *H. pylori*, the diagnosis of *H. pylori* after surgery is rather difficult, and the false positive rate tended to be higher than the normal stomach. Thus, clinicians need to understand the different efficacy of *H. pylori* diagnostic methods after gastrectomy.

Keywords

Helicobacter pylori · Diagnosis · Gastric surgery · Gastrectomy · Urea breath test

15.1 Introduction

Helicobacter pylori (*H. pylori*) eradication in patients who underwent subtotal gastrectomy for gastric cancer (GC) is strongly recommended in several guidelines [1–3] including the Maastricht VI/Florence Consensus [4]. However, there are debates about the effect and survival of *H. pylori* in the remnant stomach. Especially, as the remnant stomach after gastrectomy has a different anatomic and biological environment, the distribution of *H. pylori* becomes different or the proliferation of *H. pylori* could be scanty. Thus, there have been several studies reporting spontaneous regression of *H. pylori* after subtotal gastrectomy in peptic ulcer disease and GC patients [5–7]. These changes in postoperative *H. pylori* infection status have been suggested to be related to bile reflux and dramatic changes in acid secretion after the surgery. These cause inhibition of the growth of *H. pylori* in the remnant stomach and even spontaneous disappearance of *H. pylori* [7–10]. In addition, as there are foods in the remained stomach in spite of 6- or 8-h fasting other bacteria that produce urease cause false positive results of *H.*

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pylori, especially, in case of mucosal urease test (*Campylobacter*-like organism (CLO) test) and ^{13}C-urea breath test (^{13}C-UBT) [11, 12]. Sometimes, the higher false positive rates can make clinicians perform unnecessary *H. pylori* eradication therapy although no further current *H. pylori* infection exists. Thus, in the presence of severe intestinal metaplasia (IM), which is a hostile condition for the survival of *H. pylori*, especially with the addition of continuous bile reflux, clinicians need to be cautious about the interpretation of histology, CLOtest, or ^{13}C-UBT results and think about the "retesting" of postoperative *H. pylori* status. In this chapter, the postoperative efficacy of *H. pylori* diagnostic methods and the factors that affect these dynamic changes in *H. pylori* infection status after gastric surgery will be reviewed. In addition, the false positive test of ^{13}C-UBT before and after *H. pylori* eradication will be provided.

15.2 Dynamic Changes of *H. pylori* Status in Patients Who Underwent Gastric Cancer Surgery

Rates of *H. pylori* infection after distal gastrectomy are reported which vary widely from 20% to 70%. In general, a low prevalence of *H. pylori* is found in the remnant stomach due to decreased survivability of the microorganism in the absence of antrum. In addition, bile reflux, which frequently occurs after operation, can inhibit *H. pylori* proliferation. Thus, a high percentage of spontaneous regression and complex dynamic changes of *H. pylori* were observed after partial gastrectomy, especially in the presence of postoperative histologic IM [5] (Table 15.1).

15.2.1 Possible Mechanisms for the Dynamic Changes of *H. pylori* Status

Although the effect of subtotal gastrectomy on *H. pylori* infection status has not been fully evaluated, some reports suggested spontaneous regression of *H. pylori* after partial gastrectomy in patients with peptic ulcer disease or GC [6, 8]. Furthermore, the prevalence of *H. pylori* infection or colonization was significantly lower in the group who underwent distal gastrectomy than that of the control group in peptic ulcer patients [6]. As bile reflux is more severe in the remnant stomach after distal gastrectomy than in control, it might be the cause of the lower rate of *H. pylori* infection [7, 8]. In addition, some studies suggested that the spontaneous *H. pylori* clearance was related to the type of gastric reconstruction procedures and the time after the operation. That is, the Billroth-II procedure had a higher bile reflux rate and a lower *H. pylori* infection prevalence than the Billroth-I procedure [3]. Our team reported that spontaneous negative conversion of *H. pylori* frequently occurred in patients who had not received the eradication therapy after partial gastrectomy [5]. This is in accordance with earlier studies showing that almost 40% of patients had spontaneous regression postoperatively [7, 10]. However, *H. pylori* status could fluctuate due to the limitation of *H. pylori* tests, especially, in the background of atrophy and IM [13]. Previous studies have shown the limitations of invasive and noninvasive tests in detecting *H. pylori* infection in patients with atrophic gastritis (AG) and IM [14–16]. The bacterial load of *H. pylori* decreases as the AG and IM progresses [16], and sparse bacteria have uneven distribution in the stomach. As postoperative histology in the spontaneous negative conversion group showed more IM than the persistently positive group [5], this harsh environment of IM further kicks out the *H. pylori* spontaneously [5]. Actually, there was a report that both the degree of neutrophil infiltration and the level of COX-2 expression were significantly higher in patients with than without *H. pylori* ($p < 0.05$) [17]. This report suggests that bile acid and *H. pylori*–induced gastritis further aggravate the IM, contributing to the disappearance of *H. pylori* in the remained stomach.

15.2.2 Affecting Factors for *H. pylori* Status in Patients Who Underwent Gastric Cancer Surgery

The affecting factor for *H. pylori* status in patients who underwent GC surgery is the operation method, which determines the acid secretion, bile

Table 15.1 Comparison between persistently *H. pylori*-positive and *H. pylori*-negative conversion groups after surgery ($N = 140$) (Adapted from Yoon et al. [5])

Variable	Negative conversion ($n = 60$)*	Persistently positive ($n = 80$)	p-value
Surgery (%)			0.261
Subtotal B-I	34 (56.7)	57 (71.3)	
Subtotal B-II	20 (33.3)	18 (22.5)	
Subtotal roux-en-Y	2 (3.3)	2 (2.5)	
Subtotal with jejunal interposition	0	1 (1.3)	
Proximal gastrectomy	4 (6.7)	2 (2.5)	
Age	58.95 ± 11.09	59.09 ± 11.93	0.944
Sex (male, %)	36 (60.0)	49 (61.3)	1.000
Smoking (%)			0.168
Never	22 (36.7)	35 (43.8)	
Current	17 (28.3)	12 (15.0)	
Ex-smoker	21 (35.0)	33 (41.3)	
Alcohol (%)			0.971
None	30 (50.5)	39 (48.8)	
Social	22 (36.7)	31 (38.8)	
Heavy	8 (13.3)	10 (12.5)	
Atrophic gastritis (baseline) (%)			0.677
Negative	16 (26.7)	18 (22.5)	
Positive	38 (63.3)	50 (62.5)	
Inapplicable	6 (10.0)	12 (15.0)	
Atrophic gastritis (first follow-up) (%)			**0.008**
Negative	32 (53.3)	36 (45.0)	
Positive	8 (13.3)	20 (25.0)	
Inapplicable Not available	20 (33.3) 0	16 (20.0) 8 (10.0)	
Intestinal metaplasia (baseline) (%)			0.340
Negative	19 (31.7)	19 (23.8)	
Positive	41 (68.3)	61 (76.3)	
Intestinal metaplasia (first follow-up) (%)			**0.001**
Negative	38 (63.3)	59 (73.8)	
Positive	22 (36.7)	13 (16.3)	
Not available	0	8 (10.0)	

Statistically significant correlations ($p < 0.05$) are shown in bold style

*Spontaneous conversion to H. pylori-negative status at least once during follow-up period

reflux, and food stasis. If there is less acid secretion than normal stomach without atrophy or IM, then it is good for the proliferation of *H. pylori*. However, if there is atrophy and IM, then less acid secretion could become a bad environment for the survival of *H. pylori*. This less acid secretion and food stasis make the gastric mucosa a good environment for non-*H. pylori* microbiota. Urease-producing non-*H. pylori* microbiota will increase the false positive rate of ^{13}C-UBT or CLOtest.

15.2.2.1 Operation Methods

There are representative surgery methods depending on cancer location and early GC or advanced GC. Each method has specific characteristics in terms of distribution of *H. pylori* and non-*H. pylori* gastric microbiota depending on motility (food stasis) and the possibility of bile reflux.

1. Distal gastrectomy includes subtotal gastrectomy or radical subtotal gastrectomy. It includes Billroth II GJstomy (Gastro-jejunostomy) (Fig. 15.1a) and Roux-en-Y GJstomy (Fig. 15.1b). In the case of Roux-en-Y GJstomy, there is no bile reflux.
2. Gastro-duodenostomy. Favorable blood supply and more resistance to tension of anastomosis. However, frequently bile reflux is associated.
3. Total gastrectomy.
4. Proximal gastrectomy has esophago-gastric (E-G) stomy (gastric tube) and double tract reconstruction (Fig. 15.1c).
5. Pylorus-preserving gastrectomy. It preserves the pyloric branch of the vagus nerve but food stasis is frequent. Thus, the density and distribution of *H. pylori* in the gastric remnant could be different.

We performed a study regarding the *H. pylori* status after operation in 140 patients (mean age 59.0 years, 60.7% male) [5]. The operation methods include subtotal gastrectomy with Billroth (B)-I (65.0%), B-II (27.1%), Roux-en-Y (4.3%), jejunal interposition (0.7%), and proximal gastrectomy (4.3%), respectively. AG (62.9%) and IM (72.9%) were confirmed to be present preoperatively. The mean follow-up period between surgery and the last endoscopic follow-up was 38.0 ± 25.6 months. Of 140 patients, 80 (57.1%) were found to be persistently positive for *H. pylori* and 60 (42.9%) showed spontaneous negative conversion at least once during follow-up [5]. Among 60 patients, eight (13.3%) showed more complex postoperative dynamic changes between negative and positive results. Spontaneous negative conversion group showed a trend of having more postoperative IM compared to the persistent *H. pylori* group [5]. In this study, there was no significant difference between spontaneous conversion and persistently positive groups according to surgery type, which affects bile reflux. Instead, it suggested that IM could be a more important factor for the survival of *H. pylori* than bile reflux. However, Onoda et al. [15] reported that the prevalence of *H. pylori* infection was lower in Billroth-II reconstruction patients with severe bile reflux and subsequent stomal gastritis, suggesting a spontaneous eradication of *H. pylori* by the reflux of bile contents.

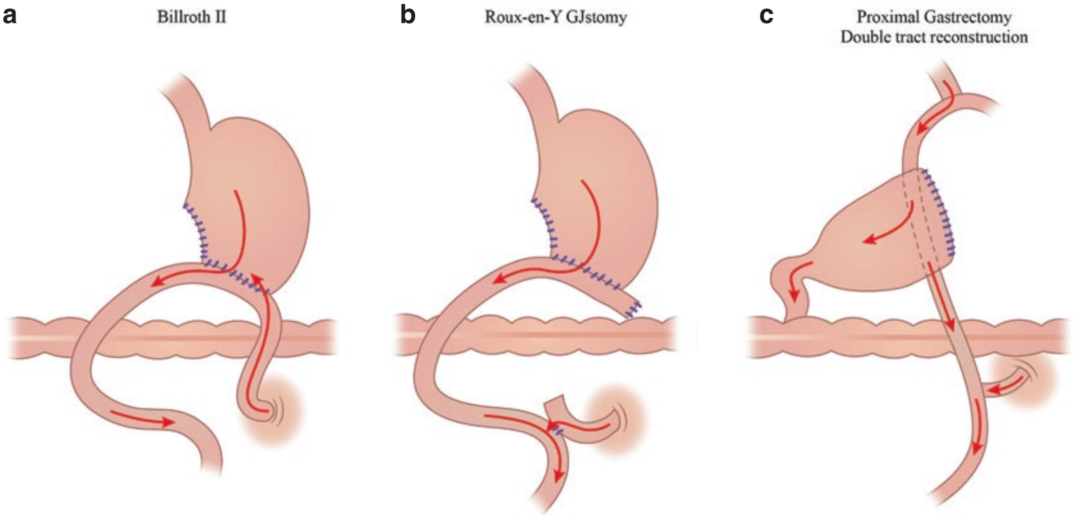

Fig. 15.1 Various reconstruction methods in stomach surgery. Distal gastrectomy includes (**a**) Billroth II GJstomy (Gastro-jejunostomy) and (**b**) Roux-en-Y GJstomy. (**c**) Double tract reconstruction is one of proximal gastrectomy methods

15.2.2.2 Bile Reflux

Subtotal gastrectomy allows bile reflux due to the absence of a pyloric sphincter. If bile reflux causes more severe gastritis, then the rate of *H. pylori* infection becomes lower [7]. For instance, a previous study reported that Roux-en-Y reconstruction after distal gastrectomy produces bile reflux, resulting in a lower rate of *H. pylori* infection [18]. In addition, there has been direct evidence that duodenogastric reflux correlated with spontaneous eradication of *H. pylori* infection following distal gastrectomy [3]. In contrast, Nakagawara et al. [19] reported that bile refluxate facilitated the survival of *H. pylori*, speculating that *H. pylori* was perhaps inhibited by other bacteria in the gut. In addition, pylorus-preserving gastrectomy for GC also resulted in significantly lower *H. pylori* prevalence after surgery [20]. Thus, the precise mechanism between bile acid reflux and *H. pylori* survival would be required for further investigation.

15.2.2.3 Postgastrectomy-Induced Hypochlorhydria

The acid secretion is very powerful in that it sterilizes the stomach inhibiting the colonization of ordinary bacteria except for *H. pylori,* which have several strong points including urease. In a hypochlorhydric state of the remnant stomach, it accelerates the colonization and the overgrowth of non-*H. pylori* urease-positive bacteria, such as *Streptococcus*, *Staphylococcus*, *Gardnerella*, *Lactococcus,* and *Enterococcus*, which could cause false positive results of rapid urease test or ^{13}C-UBT and even histology [21–24]. Furthermore, the elevation of intragastric pH does not require the neutralizing action of hydrochloric acid by *H. pylori* urease but often leads to the ultimate death of the *H pylori* as a result of overalkalization [25].

15.3 Diagnostic Methods

H. pylori infections can be diagnosed by a variety of invasive and noninvasive methods. Among these diagnostic methods, endoscopic biopsy is the gold standard. However, it is invasive and is prone to sampling error because *H. pylori* tends to be heterogeneously distributed in the remained stomach. In addition, several reports suggested that the persistence of *H. pylori* in the residual stomach decreased from 68.8% to 36% as time elapsed after surgery went from less than 1 year to more than 3 years without any eradication [26]. Taiwan's study also reported that spontaneous clearance of *H. pylori* develops in a certain number of patients who underwent distal gastrectomy [20]. In detail, there was a trend toward decreasing prevalence of *H. pylori* colonization as time after operation increased: 1–15 years, 29.5% (13/44); 16–30 years, 13.6% (3/22); and >31 years, 10% (1/10) [20]. The final spontaneous clearance rate of *H. pylori* after partial gastrectomy was 43% [20]. In addition, postgastrectomy-induced hypochlorhydria accelerates the colonization and the overgrowth of non-*H. pylori* urease-positive bacteria, resulting in false positive results. Thus, it is important to understand the false negative due to a small amount of *H. pylori* or false positive diagnosis due to the overgrowth of non-*H. pylori* urease-positive bacteria in the remained stomach.

15.3.1 Histology

Among various diagnostic methods, histology plays a pivotal role in detecting *H. pylori*, and it also provides more information about the degree of inflammation and associated pathology, such as AG, IM, and GC [27]. The diagnosis of *H. pylori* could be performed by hematoxylin and eosin (H&E) staining; however, the specificity can be improved by special stains such as modified Giemsa, Warthin-Starry silver, Genta, and immunohistochemical (IHC) stains. Thus, at least two kinds of stain methods are recommended for diagnosis in practice. H&E staining (Fig. 15.2a) is routine, and modified Giemsa stain [28] (Fig. 15.2b) seems to have an advantage over other stains because of its simplicity and consistency [27]. Warthin–Starry silver stain [29] (Fig. 15.2c) has high sensitivity, but it is time consuming that this staining method is not used in the hospital. IHC stain [30] (Fig. 15.2d) is

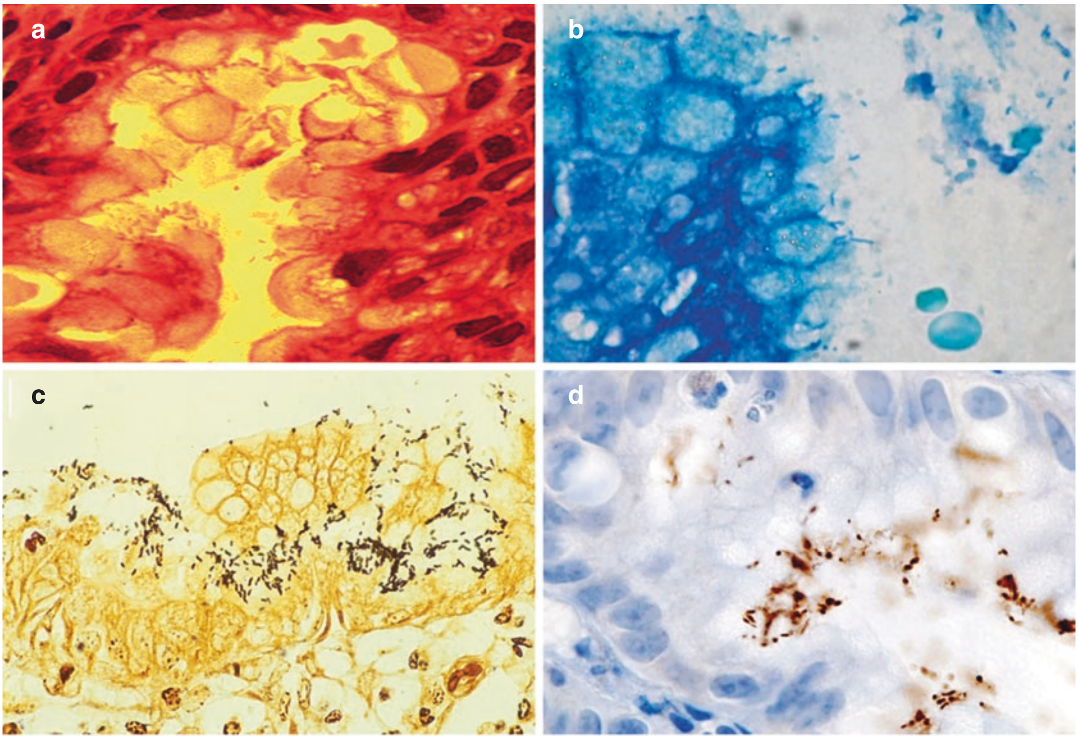

Fig. 15.2 Several methods of histological diagnosis for *Helicobacter pylori*. The spiral-shaped *Helicobacter pylori* organisms are present in hematoxylin and eosin stain (**a**), Giemsa stain (**b**), Warthin–Starry silver stain (**c**), and immunohistochemical stain (**d**) (Adapted from Lee and Kim [27])

useful in special situations but it is expensive and time consuming. In general histology has several limitations, including higher cost, longer turnaround time, dependence on the skills of the operator, and interobserver variability in assessment. Furthermore, the density of *H. pylori* can vary at different sites, possibly leading to sampling error, and the sensitivity of histology is also decreasing in patients taking proton pump inhibitors. As the number of *H. pylori* decreases by bile reflux and hypochlorhydria, it is important to perform a biopsy of several sites than just one in the normal-looking remained stomach avoiding atrophy and IM changes.

15.3.2 Rapid Urease Test

Rapid urease test such as CLOtest is frequently used in the endoscopy room because it is easy to perform, and diagnosis does not need skill or facility. As the mechanism of the rapid urease test is based on the urease activity from *H. pylori*, the density of *H. pylori* is important. However, the number of *H. pylori* decreases by bile reflux and hypochlorhydria in the remained stomach after surgery, and the sensitivity or specificity for *H. pylori* diagnosis is not as high as in the normal stomach. Thus, if the biopsy number is limited, then histology is better than the rapid mucosal urease test.

15.3.3 Serology

Serological diagnosis is a useful way to detect *H. pylori* infection because it is easy, fast, noninvasive, relatively economic, and tends to show less false negative results under certain situations, such as hemorrhagic ulcer condition or taking antibiotics and proton pump inhibitor, compared with other diagnostic methods. However, the serological diagnosis cannot differentiate the current infection from past infection. That is, it could show false positive results for several months or years because the *H. pylori* antibody persists even after *H. pylori* eradication [31]. Thus, the serological diagnosis is not recommended after antimicrobial treatment because 1 year is necessary until either the antibody is undetectable or the antibody titer is reduced to 50% after antimicrobial treatment [31]. Taken together in the case of the remnant stomach where the number of *H. pylori* decreases by bile reflux and gastrectomy-induced hypochlorhydria serology is not useful for the diagnosis of *H. pylori*.

15.3.4 ^{13}C-Urea Breath Test

The ^{13}C-UBT is a noninvasive method for diagnosing *H. pylori* infection. However, the diagnostic validity of the ^{13}C-UBT after partial gastrectomy was doubtful [11]. Generally, the ^{13}C-UBT has been reported to be one of the most accurate diagnostic tools for assessing *H. pylori* status [32, 33], and due to its speed, cost-effectiveness, and convenience, this test has been widely adopted in clinical practice [34]. After partial gastrectomy, the gastric anatomy is altered, and the test urea might be expected to pass through the stomach faster, giving different reactant percentages in reaction time, or the ^{13}C-UBT results could be influenced by bile acid reflux.

15.3.4.1 Efficacy of ^{13}C-Urea Breath Test in the Remnant Stomach After Partial Gastrectomy

Several reports have shown that ^{13}C-UBT provides lower diagnostic accuracy when using histology as a reference in the remnant stomach after partial gastrectomy [26, 35, 36]. Previously, Lotterer et al. [37] reported that the ^{13}C-UBT had shown a 100% sensitivity and 80% specificity in patients with partial stomach resection. In a Japanese study, the sensitivity, specificity, and accuracy of the ^{13}C-UBT were shown to be 96.3%, 100%, and 97.1% in the remnant stomach [38]. However, Schilling et al. [36] reported that the sensitivity of the ^{13}C-UBT was 52% and the specificity was 93%. The positive predictive value was 81.25%, the negative predictive value was 76.9% and the accuracy was 77.9% [36]. Our team also performed the ^{13}C-UBT study in 161 gastrectomzied patients [11] (Table 15.2). Our results showed that the positive ^{13}C-UBT results ($\geq 2.5‰$, $n = 47$) and negative ^{13}C-UBT results ($<2.5‰$, $n = 114$) after *H. pylori* eradication, 26 patients (16.1%), and 4 patients (2.5%) were found to show false positive and false negative results based on biopsy-based methods, respectively [11] (Fig. 15.3a). Individual values of the false positive ^{13}C-UBT results after *H. pylori* eradication (median ^{13}C-UBT value = 5.9‰) (Fig. 15.3b). Finally, the sensitivity, specificity, false positive rate, and false negative rate of the ^{13}C-UBT were 84.0%, 80.9%, 19.1%, and 16.0%, respectively. In addition, its positive and negative predictive values were 44.7% and 96.5%, thus the diagnostic validity of the ^{13}C-UBT was disappointing, especially, in the positive range of the ^{13}C-UBT results [11]. Sheu et al. [26] also reported that the sensitivity and specificity of the UBT in the gastrectomy group were lower than those in the normal group. Applying a cutoff value of 2.5%, the sensitivity and specificity were only 82.2% and 87.8%, respectively [26]. They explained that such a poor UBT diagnostic efficacy in gastrectomized patients can be attributed in part to the lower bacterial loads, inadequate coating of the stomach by urea, and disuse of test meals. However, in spite of the citric acid-test meal, the validity of ^{13}C-UBT after *H. pylori* eradication did not increase in Korea [39], which was similar to the citric acid-free meal [12]. This high false positive rate of ^{13}C-UBT could be originated from the urease-producing bacteria (*Streptococcus*, *Staphylococcus*, *Gardnerella*, *Lactococcus,* and *Enterococcus*) of which colonization and the overgrowth become possible due to hypochlorhydric state of the remnant stomach [11].

Table 15.2 Risk factors for the mismatched ^{13}C-UBT positive result compared with biopsy-based methods after *H. pylori* eradication in the partial gastrectomized patients (Adapted from Kwon et al. [11])

	^{13}C-UBT result compared with biopsy based methods			
	Matched group (*n* = 21)	Mismatched group (*n* = 26)	Univariate *p*-value	Multivariate *p*-value
Sex (male:Female)	12 (57.1%)/9 (42.9%)	17 (65.4%)/9 (34.6%)	NA	
Age (≥ 60 years old)	59.7 ± 8.9	60.5 ± 10.7	NA	
Underlying disease				
DM	5 (23.8%)	2 (7.7%)	NA	
HTN	5 (23.8%)	5 (19.2%)	NA	
Type of gastrectomy			NA	
Billoth I	10 (47.7%)	16 (61.5%)		
Billoth II	1 (4.8%)	1 (3.9%)		
Roux-en-Y	6 (28.6%)	4 (15.4%)		
Wedge resection	4 (19.0%)	4 (15.4%)		
Proximal gastrectomy	0 (0.0%)	1 (3.8%)		
Time to ^{13}C-UBT after eradication (weeks)	6.4	6.8	NA	
The mean delta value of the ^{13}C-UBT (‰)	12.3 ± 22.8	13.5 ± 15.1	NA	
The total number of *H. pylori* eradication therapies			<0.005	0.035
Single	16 (76.2%)	7 (26.9%)		
Two	3 (14.3%)	17 (65.4%)		
Three	2 (9.5%)	2 (7.7%)		
The degree of gastric mucosal atrophy in the remnant stomach			NA	
None	20 (95.2%)	20 (76.9%)		
Mild	1 (4.8%)	4 (15.4%)		
Moderate	0 (0.0%)	0 (0.0%)		
Marked	0 (0.0%)	2 (7.7%)		
The degree of gastric mucosal intestinal metaplasia in the remnant stomach			NA	
None	19 (90.5%)	16 (61.5%)		
Mild	0 (0.0%)	4 (15.4%)		
Moderate	0 (0.0%)	3 (11. 5%)		
Severe	2 (9.5%)	3 (11.5%)		

^{13}C-UBT ^{13}C-urea breath test, *NA* Not available, *DM* Diabetes mellitus, *HTN* hypertension, *H. pylori Helicobacter pylori*

15.3.4.2 Clinical Factors that Caused False Positive ^{13}C-UBT Results After *H. pylori* Eradication in the Remnant Stomach

After finding a diagnostic validity of the ^{13}C-UBT, we tried to identify clinical factors that caused false positive ^{13}C-UBT results after *H. pylori* eradication in the remnant stomach. A previous history of multiple *H. pylori* eradication therapies was found to be correlated with false positive ^{13}C-UBT results (OR 3.248; 95% CI, 1.088–9.695; $p = 0.035$) (Table 15.2) [11]. A total of 19 patients underwent second- or third-line eradication therapy for *H. pylori* infection in the ^{13}C-UBT range over 2.5‰ in mismatched group [11]. Of two patients who underwent third-line eradication, the ^{13}C-UBT values were 6‰ and

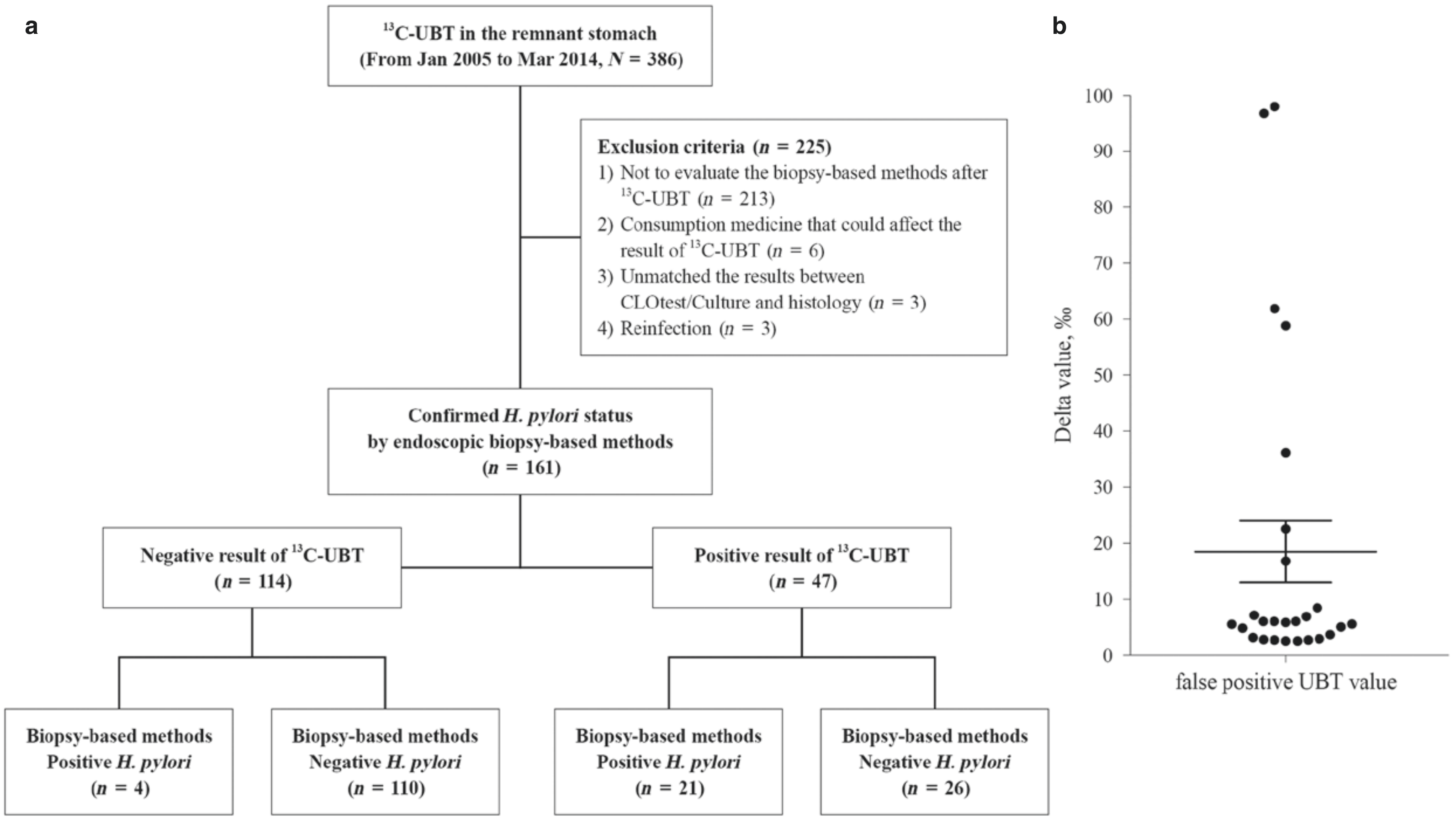
a
13C-UBT in the remnant stomach
(From Jan 2005 to Mar 2014, N = 386)
Exclusion criteria (n = 225)
1) Not to evaluate the biopsy-based methods after 13C-UBT (n = 213)
2) Consumption medicine that could affect the result of 13C-UBT (n = 6)
3) Unmatched the results between CLOtest/Culture and histology (n = 3)
4) Reinfection (n = 3)
Confirmed H. pylori status by endoscopic biopsy-based methods (n = 161)
Negative result of 13C-UBT (n = 114)
Positive result of 13C-UBT (n = 47)
Biopsy-based methods Positive H. pylori (n = 4)
Biopsy-based methods Negative H. pylori (n = 110)
Biopsy-based methods Positive H. pylori (n = 21)
Biopsy-based methods Negative H. pylori (n = 26)
b
Delta value, ‰
100
90
80
70
60
50
40
30
20
10
0
false positive UBT value

Fig. 15.3 Flow chart and the false positive ^{13}C-UBT results after *H. pylori* eradication in the partial gastrectomized patients. (**a**) Flow chart of *H. pylori* status after eradication. (**b**) Individual values of the false positive ^{13}C-UBT results after *H. pylori* eradication (median ^{13}C-UBT value = 5.9‰) (Adapted from Kwon et al. [11])

22.4‰. In these cases, delta over baselines (DOB) of the $^{13}CO_2$ were decreased compared to the first eradication therapy for *H. pylori*, but the change of DOB $^{13}CO_2$ range was variable [11]. This consequence might imply that the diagnostic accuracy of the ^{13}C-UBT is imprecise in a positive value, thus unnecessary second or third eradication could be performed according to the UBT results. This is an important point that if the clinicians intent to perform additional eradication of *H. pylori* infection, they need to be more cautious when the results fall into the low delta positive results of the ^{13}C-UBT after *H. pylori* eradication.

15.4 Conclusion

The status of *H. pylori* after partial gastrectomy shows a relatively high percentage of spontaneous regression and dynamic changes with a trend of having more histologic IM. This phenomenon is explained by the bile reflux and severe gastritis induced by *H. pylori*. In addition, postgastrectomy-induced hypochlorhydria accelerates the colonization and the overgrowth of non-*H. pylori* urease-positive bacteria, making the diagnosis of *H. pylori* becomes difficult, and false positive results frequently occur. Therefore, if the clinicians obtain a positive result of the ^{13}C-UBT after *H. pylori* eradication in partial gastrectomized patients, additional endoscopic surveillance with biopsy-based methods would be helpful to avoid unnecessary additional treatment for *H. pylori* eradication.

References

1. Fock KM, Talley N, Moayyedi P, Hunt R, Azuma T, Sugano K, et al. Asia-Pacific consensus guidelines on gastric cancer prevention. J Gastroenterol Hepatol. 2008;23:351–65.
2. Shiota S, Murakami K, Fujioka T, Yamaoka Y. Population-based strategies for *Helicobacter pylori*-associated disease management: a Japanese perspective. Expert Rev Gastroenterol Hepatol. 2010;4:149–56.
3. Fukuhara K, Osugi H, Takada N, Takemura M, Lee S, Taguchi S, et al. Duodenogastric reflux eradicates *Helicobacter pylori* after distal gastrectomy. Hepato-Gastroenterology. 2004;51:1548–50.
4. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection—the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
5. Yoon K, Kim N, Kim J, Lee JW, Lee HS, Lee JC, et al. Dynamic changes in *Helicobacter pylori* status following gastric cancer surgery. Gut Liver. 2017;11:209–15.
6. Bair MJ, Wu MS, Chang WH, Shih SC, Wang TE, Chen CJ, et al. Spontaneous clearance of *Helicobacter pylori* colonization in patients with partial gastrectomy: correlates with operative procedures and duration after operation. J Formos Med Assoc. 2009;108:13–9.
7. Abe H, Murakami K, Satoh S, Sato R, Kodama M, Arita T, et al. Influence of bile reflux and *Helicobacter pylori* infection on gastritis in the remnant gastric mucosa after distal gastrectomy. J Gastroenterol. 2005;40:563–9.
8. Li XB, Lu H, Chen HM, Chen XY, Ge ZZ. Role of bile reflux and *Helicobacter pylori* infection on inflammation of gastric remnant after distal gastrectomy. J Dig Dis. 2008;9:208–12.
9. Johannesson KA, Hammar E, Staël von Holstein C. Mucosal changes in the gastric remnant: long-term effects of bile reflux diversion and *Helicobacter pylori* infection. Eur J Gastroenterol Hepatol. 2003;15:35–40.
10. Suh S, Nah JC, Uhm MS, Jung YM, Kim N, Lee DH, et al. Changes in prevalence of *Helicobacter pylori* infection after subtotal gastrectomy. Hepato-Gastroenterology. 2012;59:646–8.
11. Kwon YH, Kim N, Lee JY, Choi YJ, Yoon K, Yoon H, et al. The diagnostic validity of the ^{13}C-urea breath test in the gastrectomized patients: single tertiary center retrospective cohort study. J Cancer Prev. 2014;19:309–17.
12. Kwon YH, Kim N, Lee JY, Choi YJ, Yoon K, Hwang JJ, et al. The diagnostic validity of citric acid-free, high dose (13) C-urea breath test after *Helicobacter pylori* eradication in Korea. Helicobacter. 2015;20:159–68.
13. Kim SE, Park YS, Kim N, Kim MS, Jo HJ, Shin CM, et al. The effect of *Helicobacter pylori* eradication on functional dyspepsia. J Neurogastroenterol Motil. 2013;19:233–43.
14. Korstanje A, van Eeden S, Offerhaus GJ, Sabbe LJM, den Hartog F, Biemond I, et al. The 13carbon urea breath test for the diagnosis of *Helicobacter pylori* infection in subjects with atrophic gastritis: evaluation in a primary care setting. Aliment Pharmacol Ther. 2006;24:643–50.
15. Onoda N, Maeda K, Sawada T, Wakasa K, Arakawa T, Chung KH. Prevalence of *Helicobacter pylori* infection in gastric remnant after distal gastrectomy for primary gastric cancer. Gastric Cancer. 2001;4:87–92.
16. Kang HY, Kim N, Park YS, Hwang JH, Kim JW, Jeong SH, et al. Progression of atrophic gastritis and

intestinal metaplasia drives *Helicobacter pylori* out of the gastric mucosa. Dig Dis Sci. 2006;51:2310–5.

17. Fukuhara K, Osugi H, Takada N, Takemura M, Lee S, Morimura K, et al. Effect of *H. pylori* on COX-2 expression in gastric remnant after distal gastrectomy. Hepato-Gastroenterology. 2004;51:1515–8.
18. Chan DC, Fan YM, Lin CK, Chen CJ, Chen CY, Chao YC. Roux-en-Y reconstruction after distal gastrectomy to reduce enterogastric reflux and *Helicobacter pylori* infection. J Gastrointest Surg. 2007;11:1732–40.
19. Nakagawara H, Miwa K, Nakamura S, Hattori T. Duodenogastric reflux sustains *Helicobacter pylori* infection in the gastric stump. Scand J Gastroenterol. 2003;38:931–7.
20. Miyashita T, Miwa K, Inokuchi M, Nakagawara H, Tajima H, Takamura H, et al. Spontaneous clearance of *Helicobacter pylori* after pylorus-preserving gastrectomy for gastric cancer. Oncol Rep. 2013;30:299–303.
21. Brandi G, Biavati B, Calabrese C, Granata M, Nannetti A, Mattarelli P, et al. Urease-positive bacteria other than *Helicobacter pylori* in human gastric juice and mucosa. Am J Gastroenterol. 2006;101:1756–61.
22. Gurbuz A, Ozel A, Narin Y, Yazgan Y, Baloglu H, Demirturk L. Is the remarkable contradiction between histology and ^{14}C urea breath test in the detection of *Helicobacter pylori* due to false-negative histology or false-positive ^{14}C urea breath test? J Int Med Res. 2005;33:632–40.
23. Osaki T, Mabe K, Hanawa T, Kamiya S. Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of *Helicobacter pylori* infection. J Med Microbiol. 2008;57:814–9.
24. Slomianski A, Schubert T, Cutler AF. ^{13}C urea breath test to confirm eradication of *Helicobacter pylori*. Am J Gastroenterol. 1995;90:224–6.
25. Bell NJ, Hunt RH. Progress with proton pump inhibition. Yale J Biol Med. 1992;65:649–57; discussion 689–92.
26. Sheu BS, Lee SC, Lin PW, Wang ST, Chang YC, Yang HB, et al. Carbon urea breath test is not as accurate as endoscopy to detect *Helicobacter pylori* after gastrectomy. Gastrointest Endosc. 2000;51:670–5.
27. Lee JY, Kim N. Diagnosis of *Helicobacter pylori* by invasive test: histology. Ann Transl Med. 2015;3:10.
28. Uthman E. *Helicobacter pylori*, Gastric Mucosa, Giemsa stain.jpg. https://commons.wikimedia.org/wiki/File:Helicobacter_pylori,_Gastric_Mucosa,_Giemsa_stain_(390307643).jpg. Accessed 9 Feb 2023.
29. Tsutsumi Y. Pylorigastritis.jpg. https://commons.wikimedia.org/wiki/File:Pylorigastritis.jpg. Accessed 9 Feb 2023.
30. KGH. Immunohistochemical detection of *Helicobacter pylori* histopathology.jpg. https://commons.wikimedia.org/wiki/File:Immunohistochemical_detection_of_Helicobacter_pylori_histopathology.jpg. Accessed 9 Feb 2023.
31. Lee JH, Kim N, Chung JI, Kang KP, Lee SH, Park YS, et al. Long-term follow-up of *Helicobacter pylori* IgG serology after eradication and reinfection rate of *H. pylori* in South Korea. Helicobacter. 2008;13:288–94.
32. Bazzoli F, Zagari M, Fossi S, Pozzato P, Ricciardiello L, Mwangemi C, et al. Urea breath tests for the detection of *Helicobacter pylori* infection. Helicobacter. 1997;2:34–7.
33. Savarino V, Vigneri S, Celle G. The ^{13}C urea breath test in the diagnosis of *Helicobacter pylori* infection. Gut. 1999;45:18–22.
34. Savarino V, Mela GS, Zentilin P, Bisso G, Pivari M, Mansi C, et al. Comparison of isotope ratio mass spectrometry and nondispersive isotope-selective infrared spectroscopy for ^{13}C-urea breath test. Am J Gastroenterol. 1999;94:1203–8.
35. Adamopoulos AB, Stergiou GS, Sakizlis GN, Tiniakos DG, Nasothimiou EG, Sioutis DK, et al. Diagnostic value of rapid urease test and urea breath test for *Helicobacter pylori* detection in patients with Billroth II gastrectomy: a prospective controlled trial. Dig Liver Dis. 2009;41:4–8.
36. Schilling D, Jakobs R, Peitz U, Sulliga M, Stolte M, Riemann J, et al. Diagnostic accuracy of 13C-urea a breath test in the diagnosis of *Helicobacter pylori* infection in patients with partial gastric resection due to peptic ulcer disease. Digestion. 2001;63:8–13.
37. Lotterer E, Lüdtke FE, Tegeler R, Lepsien G, Bauer FE. The ^{13}Curea breath test—detection of *Helicobacter pylori* infection in patients with partial gastrectomy. Z Gastroenterol. 1993;31:115–9.
38. Kubota K, Shimoyama S, Shimizu N, Noguchi C, Mafune K, Kaminishi M, et al. Studies of ^{13}C-urea breath test for diagnosis of *Helicobacter pylori* infection in patients after partial gastrectomy. Digestion. 2002;65:82–6.
39. Kwon YH, Kim N, Yoon H, Shin CM, Park YS, Lee DH. Effect of citric acid on accuracy of ^{13}C-urea breath test after *Helicobacter pylori* eradication therapy in a region with a high prevalence of atrophic gastritis. Gut Liver. 2019;13:506–14.

Part IV

Symptom

Symptoms of Acute and Chronic *H. pylori* Infection

16

Nayoung Kim

Abstract

Helicobacter pylori (*H. pylori*) has urease and *ureI* gene to overcome gastric acid to survive on the acidic human stomach mucous membrane. Once *H. pylori* enters the stomach, acute gastroenteritis symptoms, such as nausea, vomiting, epigastric pain, and heartburn, occur. These symptoms emerge within the first week of infection, and the symptoms reach their maximum severity on the ninth to 12th day of the infection. Then, the symptoms get better, and most of those are gone after 2 weeks of the infection. Based on a gastroscopic view of *H. pylori* infection, erythema can be seen on the gastric mucosa after the fifth day of infection, and gastric juice pH is reported to be 7.0, which is neutral. Then, the erythema findings get better after 2 weeks of infection, while the gastric juice pH is 7.5, which is indifferent. Later, the gastroscopic view is back to normal after 74 days of *H. pylori* infection, while lymphocyte and plasma cell infiltrations increase, and gastric juice pH becomes 2. Thus, continued *H. pylori* infection after 2 months can be considered as chronic infection. Once chronic infection is maintained, *H. pylori* influences on symptoms that might be related to the brain-gut axis through various immunologic mechanisms and is expected to cause symptoms on organs other than stomach. However, unequivocal data concerning the direct and immediate effect of *H. pylori* infection on the brain-gut axis are still lacking. Therefore, further studies evaluating the clinical importance of these host-bacteria interactions will improve our understanding of *H. pylori* infection pathophysiology.

Keywords

Symptom · Acute · Chronic · Infection · *Helicobacter pylori* · Brain-gut axis

16.1 Introduction

Helicobacter pylori (*H. pylori*) is thought to have evolved continuously inside the human stomach when mankind migrated from Africa to America and Oceania [1]. *H. pylori* is a gram-negative bacteria that has urease, which takes up 10% of the bacteria's entire protein, and *ureI* gene to overcome gastric acid and to survive on the human gastric mucosa that is covered by gastric acid. In other words, *H. pylori* has gone through successful complex mutations [2, 3]. *H. pylori* is assumed to be helpful for the mankind, since it

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has existed for a long time with the human [4]. However, these bacteria infect 50% of global population and induce severe gastric disorders on 10% of those infected people [5–7]. Once *H. pylori* enters the stomach, acute gastroenteritis symptoms, such as nausea, vomiting, epigastric pain, and heartburn occur and stomach pH increases abruptly. Furthermore, intermittent or persistent pain or discomfort in the upper abdomen or lower part of the chest, and a feeling of postprandial fullness develop, whereas less common symptoms include vomiting, diarrhea, belching, and headache [8]. Most of these symptoms are gone when the bacteria succeed to survive and become a chronic infection. Nonetheless, there have been reports recently that *H. pylori* infection variously affects organs other than the stomach. Thus, researches on the brain-gut axis have been conducted in full scale. This chapter is going to introduce initial symptoms when *H. pylori* enters the stomach, overcome processes of *H. pylori* and **s**ymptoms of chronic *H. pylori* infection.

16.2 The Induction Mechanisms of Symptoms After *H. pylori* Infection

When *H. pylori* enters the stomach of a human host and colonizes itself on gastric mucosa, heavy neutrophils infiltrate into the mucosa. Neutrophil infiltration and the severity of gastric mucosa damage are closely related, and it seems like gastric epithelial cell damage is caused by infiltrated leukocytes due to *H. pylori* infection [9]. A protein on *H. pylori* surface called *H. pylori* neutrophil-activating protein (HP-NAP) attracts neutrophils from blood to infected gastric mucosa and activates them [10–12]. Other than HP-NAP, urease that helps *H. pylori* to survive in acidic condition of stomach attracts neutrophils and monocytes and affects on inflammatory cytokine secretion [13] (Fig. 16.1). Also, interleukin (IL)-1β, IL-2, IL-6, and IL-8 and tumor necrosis factor (TNF)-α increase under *H. pylori* infection; IL-8 is the most powerful neutrophil-activating protein, and it is more active when *H. pylori* has *cag*-pathogenicity island (PAI) [12]. *H. pylori* infection induces active immunologic reactions on overall body or on local area, but these reactions cannot eradicate *H. pylori* and rather contribute to stomach cell damage. Th2 reaction is expected because *H. pylori* does not infiltrate into stomach cell by itself, but *H. pylori* infection induces IL-18 production, which leads to Th1 reaction, and Th1 reaction helps *H. pylori* for its sustained survival in stomach [12]. Meanwhile, there is an argument that sialic acid-binding adhesion (SabA) factor of *H. pylori* attaches to sialylated glycoconjugates on the surface of neutrophil, instead of HP-NAP, to activate neutrophil and induces acute gastroenteritis [14]. If *H. pylori* infection continues, T cell, B cell, plasma cell, and macrophage as well as neutrophil infiltrate and induce gastric epithelial cell damage [12]. Thus, these reactions contribute to acute and chronic inflammations due to *H. pylori*, and a recent research suggests that the relationship between *H. pylori* and the brain-gut axis induces the symptoms [15]. In other words, cytotoxins that are similar to VacA of *H. pylori* and IL-8, neuro-inflammation due to HP-NAP, and free radical that is derived by *H. pylori* are assumed to damage the blood-brain barrier and cause alteration of neurotransmitter secretion of the gastric mucosa and spinal cord, and the changes of stomach microbiota could be the causes of gastroenterological symptoms after *H. pylori* infection [15] (Fig. 16.2).

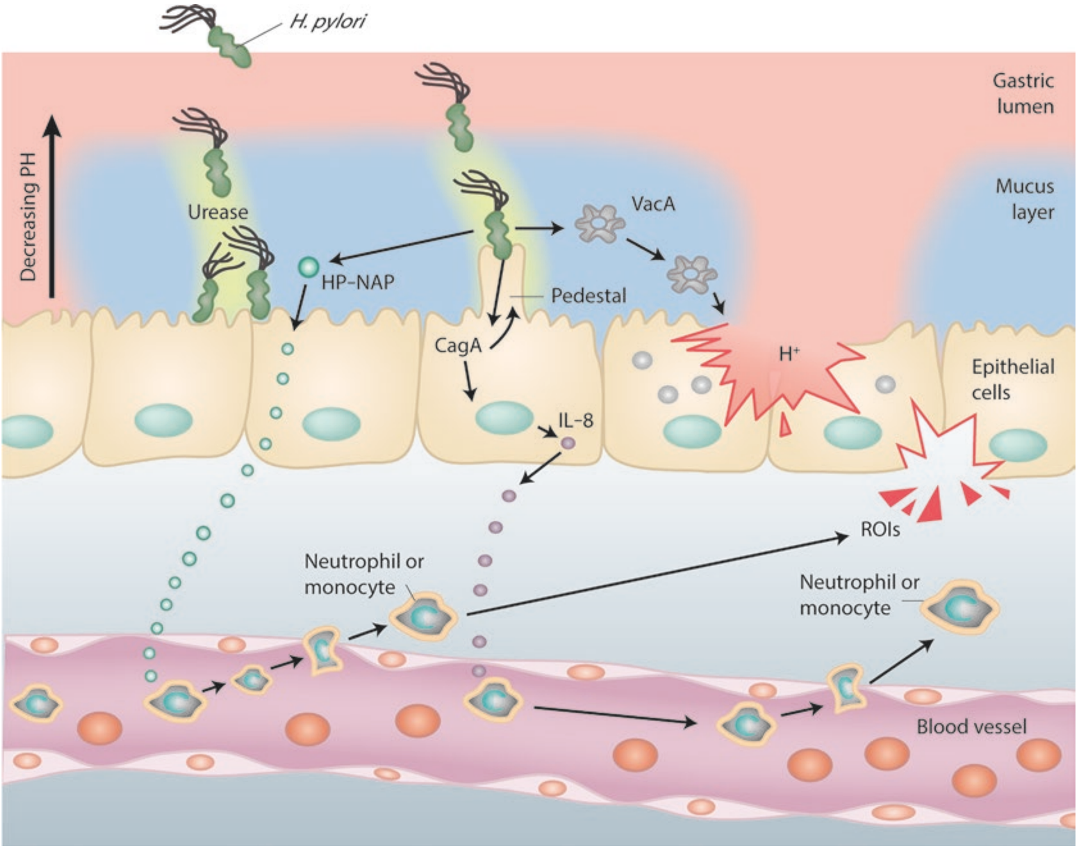
H. pylori
Gastric lumen
Mucus layer
Decreasing PH
Urease
VacA
HP-NAP
Pedestal
CagA
H⁺
Epithelial cells
IL-8
ROIs
Neutrophil or monocyte
Neutrophil or monocyte
Blood vessel

Fig. 16.1 Main virulence factors that induce diseases and help *H. pylori* surviving on the stomach mucous membrane. *HP-NAP, H. pylori* neutrophil-activating protein; *ROIs,* reactive oxygen intermediates (Adapted from Montecucco and Rappuoli [13])

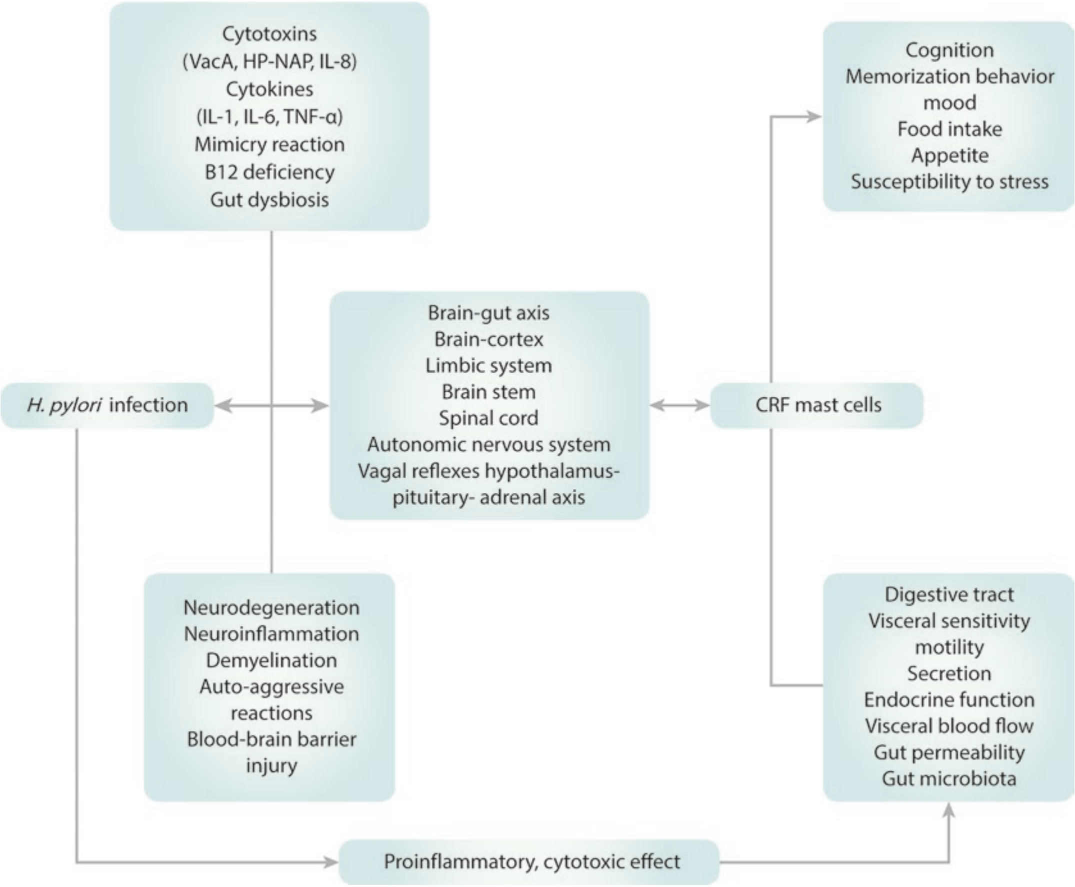
Cytotoxins
(VacA, HP-NAP, IL-8)
Cytokines
(IL-1, IL-6, TNF-α)
Mimicry reaction
B12 deficiency
Gut dysbiosis
Cognition
Memorization behavior
mood
Food intake
Appetite
Susceptibility to stress
H. pylori infection
Brain-gut axis
Brain-cortex
Limbic system
Brain stem
Spinal cord
Autonomic nervous system
Vagal reflexes hypothalamus-pituitary- adrenal axis
CRF mast cells
Neurodegeneration
Neuroinflammation
Demyelination
Auto-aggressive reactions
Blood-brain barrier injury
Digestive tract
Visceral sensitivity
motility
Secretion
Endocrine function
Visceral blood flow
Gut permeability
Gut microbiota
Proinflammatory, cytotoxic effect

Fig. 16.2 Induction of gastroenterological symptoms that are related to the brain-gut axis due to *H. pylori* infection. *CRF* Corticotropin-releasing factor, *TNF-α* Tumor necrosis factor-alpha, *IL* Interleukin, *HP-NAP H. pylori* neutrophil-activating protein, (Adapted from Budzynski and Klopocka [15])

16.3 Brain-Gut Axis, a Possible Mechanism of Symptoms of *H. pylori* Infection

Clinical manifestations are nonspecific, and some may be justified only by the presence of complications. Frequent symptoms such as nausea or epigastric pain may decrease in frequency and intensity or even disappear with or without the eradication of the bacteria [16]. A meta-analysis documented a statistically significant association with epigastric pain but no statistically significant association with other nonspecific digestive symptoms, such as vomiting, diarrhea, flatulence, abdominal pain, or constipation [17]. The fact that only 10–15% of *H. pylori*-infected patients presenting dyspeptic symptoms [18] suggest a role for other individual factors, including nervous system imbalance, as an indispensable cofactor in gastritis or ulcer disease pathogenesis [15]. These arguments imply that *H. pylori* infection may induce changes in the function and morphology of the digestive tract both directly through cytotoxin (CagA, VacA) release and inflammatory process activation and, indirectly, via the brain-gut axis [19]. The brain-gut axis integrates the central, peripheral, enteric, and autonomic nervous systems, as well as the endocrine and immunological systems, with gastrointestinal (GI) functions and environmental stimuli, including gastric and intestinal microbiota [15]. As *H. pylori* infection causes very active inflammation in the stomach, this process of acute infection may also induce abnormalities indirectly by affecting the brain-gut axis, similar to other microorganisms present in the alimentary tract [15]. The bidirectional relationship between *H. pylori* infection and the brain-gut axis influences both the contagion process and the host's neuroendocrine-immunological reaction to it, resulting in alterations in cognitive functions, food intake and appetite, immunological response, and modification of symptom sensitivity thresholds [15]. Furthermore, disturbances in the digestive tract permeability, motility, and secretion can occur, similar to a form of irritable bowel syndrome. Actually *H. pylori* has been an important candidate for etiological factor of functional dyspepsia (FD) [20]. *H. pylori* infection could contribute to the generation of dyspepsia or gastric symptoms by affecting the brain-gut axis which is involved in bacterial toxins, *H. pylori*-induced cytokines, or gastric neurotransmitter [15]. For instance, *H. pylori*-related gastritis may impede the production of ghrelin by causing destruction of ghrelin-producing cells [21, 22]. Choi et al. suggested that acyl ghrelin may be associated with appearance and exacerbation of postprandial fullness or early satiation probably related with the impaired gastric motility [23]. This is supported by the results of Shindo et al. in which postprandial discomfort syndrome (PDS)-subtype FD patients showed reduced acyl ghrelin levels correlated with delayed gastric emptying [24]. Furthermore, a recent study demonstrated that ghrelin agonist accelerated gastric emptying [25]. Actually anti-*H. pylori* therapy enhanced acyl ghrelin levels following an improvement of atrophy and correlated with improvement of PDS symptoms [23]. Similarly FD symptoms disappeared following *H. pylori* eradication in part of FD patients [26–28]. Finally, *H. pylori*-associated FD has been now accepted as one entity which is different from idiopathic FD [29]. From this background, *H. pylori* may have some relationship with the brain-gut axis through activation of neurogenic inflammatory processes or by microelement deficiency secondary to functional and morphological changes in the digestive tract. In addition, *H. pylori* can alter signaling in the brain-gut axis by mast cells, the main brain-gut axis effector, as *H. pylori* infection is associated with change of mast cell infiltration in the digestive tract [15].

16.4 Symptoms and Endoscopic and Histological Findings of Acute *H. pylori* Infection

Symptoms of acute *H. pylori* infection are thought to appear on 60% of entire infected patients [30]. Once the bacteria enter the stomach and induce infection, subjects developed variable GI and constitutional symptoms. Dyspeptic symptoms were mild to moderate in severity and typically appeared during the first week after challenge and peaked between days 9 and 12 [31]. Other recorded symptoms were headache ($n = 1$), anorexia ($n = 1$), dyspepsia ($n = 7$), vomiting ($n = 1$), abdominal pain ($n = 6$), belching ($n = 5$), and halitosis ($n = 3$). These symptoms start within the first week of infection, and these symptoms reach their maximum degrees on 9–12 days after the infection [31]. Then, the symptoms are attenuated by themselves, and most of the symptoms are gone after 2 weeks of the infection [31–34]. In no instance did symptoms interfere with regular activities of daily living [31]. In the different group, symptoms typically first occurred sporadically after day 3 after challenge with 10^4–10^{10} colony-forming unit (CFU) of a *cag* PAI negative, outer membrane inflammatory protein (OipA)-positive strain of *H. pylori*, peaked in the second week, and remained relatively consistent for 1 week and then resolved [31]. Again, early symptoms included nausea and abdominal cramps, followed by feelings of abdominal fullness, belching, and mild decreased appetite after 2 weeks [31]. Other reports also described similar symptoms [32–34]. Detachment of gastric epithelial cells and multiple neutrophils on the surface of the lamina propria was observed through histological examination after the fifth day of infection in the antrum and the corpus of the stomach [31]. Erythema can be seen on gastric mucosa after the fifth day of infection via gastroscopy, and the pH of gastric juice is reported to be

7.0, which is neutral [30]. However, the erythema gets better after 2 weeks of infection, but the gastric juice pH was still 7.5 [30]. Histological examination after 2 weeks of infection showed similar observations to that of the fifth day of the infection, but the number of chronic inflammatory cells increased and showed the characteristics of both acute and chronic gastritis [31]. Also, gastroscopic observation after the 74th day of infection showed normal condition, and gastric juice pH was 2, which is acidic [31]. A histological examination at this time showed that the number of lymphocytes and plasma cells increased on both the antrum and corpus of the stomach, and lymphoid follicle formations were confirmed, which indicate chronic gastritis [31]. Graham et al. also measured mucosal cytokine levels [31]. That is, IL-1β, IL-8, and IL-6 levels increased significantly 2 weeks after inoculation (IL-1β 9.2–49.1 pg/mg protein ($p = 0.05$); IL-6 0.0–0.14 pg/mg protein ($p = 0.005$); IL-8 4.1–179.4 pg/mg protein ($p = 0.005$)) [31]. IL-8 levels increased more than 20-fold in specimens obtained 2 weeks after inoculation. In corporal biopsy specimens, there was only a significant increase in IL-8 levels (4.6–96.9 pg/mg protein) ($p = 0.05$) [15]. After 12 weeks, IL-8 levels tended to decrease compared with 2 weeks, especially in the antral specimens, but IL-1β levels were not significantly different between 2 and 12 weeks [31].

Prevalent gastroenterological conditions and symptoms during the 12-month baseline period included abdominal pain/tenderness (59.3%), gastroesophageal reflux disease (49.2%), gastritis (39.4%), and dyspepsia (30.5%) [8]. Furthermore, resolution (and likewise, persistence) of symptoms does not correlate with the success versus failure of eradication treatment and thus is not a replacement for nonserology posteradication treatment testing [35].

16.5 Symptoms of Chronic *H. pylori* Infection

H. pylori constantly gets used to its host environment and continuously affects on the host to induce symptoms as long as it survives. Chronic inflammation induces atrophic gastritis and intestinal metaplasia on gastric epithelial cells and causes abnormal physiologic activities along with its relationship with the brain-gut axis, so functional dyspepsia could be induced as a result [15] (Fig. 16.2). As atrophic gastritis and intestinal metaplasia frequently develop in the elderly, there was an article focusing on *H. pylori* infection in the elderly [36]. An Italian study showed that about 40% of more than 3000 people over 60 years old had GI symptoms due to the presence of disordered digestive function in the elderly population [37]. The functional decline of the upper GI tract is mainly characterized by the atrophic gastric mucosa and reduced digestive enzyme activity, and infection (e.g., *H. pylori*), nonsteroidal anti-inflammatory drugs (NSAIDs), and other factors can lead to the further deterioration [38].

16.5.1 Brain-Gut Axis Relationship with Chronic *H. pylori* Infection

The brain-gut axis relationship with chronic *H. pylori* infection has been suspected to induce various clinical symptoms due to (1) changes of dietary pattern [39, 40]; (2) amnestic and cognitive disorder [39–41]; depression and anxiety [40]; (3) hormone alterations, such as substance P, somatostatin, vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP) [42], gastrin, and neuropeptides leptin and ghrelin [43, 44]; (4) effects on autonomic nervous system balance and vagus nerve reflection [45]; (5) abnormal GI tract movement [46]; (6) visceral nerve hypersensitivity to chemo- and mechanostimulants [47, 48]; (7) abnormal gastric secretion [46, 49]; (8) increase of intestinal permeability [50, 51]; and (9) effects on intestinal microbiota [52, 53] (Fig. 16.2). Moreover, *H. pylori* infection is associated with the upregulation of toll-like receptors and cytokine overproduction, especially TNF-α, IL-1, IL-6, and IL-8 [54–56], thereby indirectly influencing the brain-gut axis. These immune mediators may stimulate mast cells in the gastric mucosa, as well as the hypothalamus and brain stem (via neuroendocrine-immune crosstalk) [57, 58], thereby activating the sympathetic autonomic nervous system (ANS) and pituitary-suprarenal axis, resulting in increased cortisol and adrenalin secretion [51, 57–59]. These associations could be quite exaggerated, but it is also true that the normalization of some of the symptoms has been observed after *H. pylori* eradication [23, 26–28, 45, 60–65], so chronic *H. pylori* infection can explain the symptoms of both inside and outside of digestive system.

16.5.2 Extragastric Diseases of Chronic *H. pylori* Infection

Several papers related to the topic of extragastric disorders of *H. pylori* infection have been published in recent decades, including hematological, metabolic, cardiovascular, neurodegenerative, and allergy illnesses [66–70]. In most cases, extragastric manifestations are due to systemic subclinical inflammation caused by *H. pylori*, which is why early eradication may limit any unfavorable effects associated with the onset of these symptoms [67]. Therefore, *H. pylori* eradication gets more important in terms of gastric cancer prevention and treating symptoms of the inside and the outside of the digestive system. However, clear explanations are still lacking, and more researches about various mechanisms of symptoms due to chronic *H. pylori* infection are needed.

16.6 Conclusions

H. pylori is the only bacteria that colonize and survive on the human stomach, although the host produces various immunologic reactions. Acute *H. pylori* infection can be seen from 60% of infected patients and induces symptoms of headache, nausea, epigastric pain, and body aches. Then, the infection becomes a chronic state after 2 months of *H. pylori* infection. As *H. pylori* keeps getting used to its host environment, these bacteria constantly affect on the host to induce symptoms. In other words, the chronic infection causes not only atrophic gastritis and intestinal metaplasia but also visceral hypersensitivity, abnormal gastric secretions, and abnormal GI movement. Other than that, *H. pylori* could influence on symptoms that are related to the brain-gut axis through various immunologic mechanisms. An awareness of *H. pylori* and associated health hazards is necessary to minimize or even eradicate the infection [71].

References

1. Moodley Y, Linz B, Bond RP, Nieuwoudt M, Soodyall H, Schlebusch CM, et al. Age of the association between *Helicobacter pylori* and man. PLoS Pathog. 2012;8:e1002693.
2. Peek RM Jr, Blaser MJ. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. Nat Rev Cancer. 2002;2:28–37.
3. Linz B, Balloux F, Moodley Y, Manica A, Liu H, Roumagnac P, et al. An African origin for the intimate association between humans and *Helicobacter pylori*. Nature. 2007;445:915–8.
4. Devi SM, Ahmed I, Khan AA, Rahman SA, Alvi A, Sechi LA, et al. Genomes of *Helicobacter pylori* from native Peruvians suggest admixture of ancestral and modern lineages and reveal a western type *cag*-pathogenicity Island. BMC Genomics. 2006;7:191.
5. Yamaoka Y. *Helicobacter pylori* typing as a tool for tracking human migration. Clin Microbiol Infect. 2009;15:829–34.
6. Ching CK, Wong BC, Kwok E, Ong L, Covacci A, Lam SK. Prevalence of CagA bearing *Helicobacter pylori* strains detected by the anti-CagA assay in patients with peptic ulcer disease and in controls. Am J Gastroenterol. 1996;91:949–53.
7. Weel JF, van der Hulst RW, Gerrits Y, Roorda P, Feller M, Dankert J, et al. The interrelationship between cytotoxin-associated gene A, vacuolating cytotoxin, and *Helicobacter pylori*-related diseases. J Infect Dis. 1996;173:1171–5.
8. Gebeyehu E, Nigatu D, Engidawork E. Complete symptom resolution as predictor of *Helicobacter pylori* eradication and factors affecting symptom resolution: prospective follow up study. PLoS One. 2021;16:e0246624.
9. Dundon WG, Nishioka H, Polenghi A, Papinutto E, Zanotti G, Montemurro P, et al. The neutrophil-activating protein of *Helicobacter pylori*. Int J Med Microbiol. 2002;291:545–50.
10. Evans DJ Jr, Evans DG, Takemura T, Nakano H, Lampert HC, Graham DY, et al. Characterization of a *Helicobacter pylori* neutrophil-activating protein. Infect Immun. 1995;63:2213–20.

11. Satin B, Del Giudice G, Della Bianca V, Dusi S, Laudanna C, Tonello F, et al. The neutrophil-activating protein (HP-NAP) of *Helicobacter pylori* is a protective antigen and a major virulence factor. J Exp Med. 2000;191:1467–76.
12. Suerbaum S, Michetti P. *Helicobacter pylori* infection. N Engl J Med. 2002;347:1175–86.
13. Montecucco C, Rappuoli R. Living dangerously: how *Helicobacter pylori* survives in the human stomach. Nat Rev Mol Cell Biol. 2001;2:457–66.
14. Unemo M, Aspholm-Hurtig M, Ilver D, Bergström J, Borén T, Danielsson D, et al. The sialic acid binding SabA adhesin of *Helicobacter pylori* is essential for nonopsonic activation of human neutrophils. J Biol Chem. 2005;280:15390–7.
15. Budzyński J, Klopocka M. Brain-gut axis in the pathogenesis of *Helicobacter pylori* infection. World J Gastroenterol. 2014;20:5212–25.
16. Marginean CM, Cioboata R, Olteanu M, Vasile CM, Popescu M, Popescu AIS, et al. The importance of accurate early diagnosis and eradication in *Helicobacter pylori* infection: pictorial summary review in children and adults. Antibiotics (Basel). 2022;12:60.
17. Spee LAA, Madderom MB, Pijpers M, Van Leeuwen Y, Berger MY. Association between *Helicobacter pylori* and gastrointestinal symptoms in children. Pediatrics. 2010;125:e651–69.
18. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of *Helicobacter pylori* infection—the Maastricht IV/ Florence consensus report. Gut. 2012;61:646–64.
19. Goodwin RD, Talley NJ, Hotopf M, Cowles RA, Galea S, Jacobi F. A link between physician-diagnosed ulcer and anxiety disorders among adults. Ann Epidemiol. 2013;23:189–92.
20. Suzuki H, Moayyedi P. *Helicobacter pylori* infection in functional dyspepsia. Nat Rev Gastroenterol Hepatol. 2013;10:168–74.
21. Suzuki H, Masaoka T, Hosoda H, Ota T, Minegishi Y, Nomura S, et al. *Helicobacter pylori* infection modifies gastric and plasma ghrelin dynamics in Mongolian gerbils. Gut. 2004;53:187–94.
22. Isomoto H, Ueno H, Saenko VA, Mondal MS, Nishi Y, Kawano N, et al. Impact of *Helicobacter pylori* infection on gastric and plasma ghrelin dynamics in humans. Am J Gastroenterol. 2005;100:1711–20.
23. Choi YJ, Kim N, Yoon H, Shin CM, Park YS, Park JH, et al. Increase in plasma acyl ghrelin is associated with amelioration of dyspepsia following *Helicobacter pylori* eradication. J Gastroenterol. 2016;51:548–59.
24. Shindo T, Futagami S, Hiratsuka T, Horie A, Hamamoto T, Ueki N, et al. Comparison of gastric emptying and plasma ghrelin levels in patients with functional dyspepsia and non-erosive reflux disease. Digestion. 2009;79:65–72.
25. Shin A, Camilleri M, Busciglio I, Burton D, Smith SA, Vella A, et al. The ghrelin agonist RM-131 accelerates gastric emptying of solids and reduces symptoms in patients with type 1 diabetes mellitus. Clin Gastroenterol Hepatol. 2013;11:1453–9. e4.
26. Kim SE, Park YS, Kim N, Kim MS, Jo HJ, Shin CM, et al. The effect of *Helicobacter pylori* eradication on functional dyspepsia. J Neurogastroenterol Motil. 2013;19:233–43.
27. Jin X, Li YM. Systematic review and meta-analysis from Chinese literature: the association between *Helicobacter pylori* eradication and improvement of functional dyspepsia. Helicobacter. 2007;12:541–6.
28. Zhao B, Zhao J, Cheng WF, Shi WJ, Liu W, Pan XL, et al. Efficacy of *Helicobacter pylori* eradication therapy on functional dyspepsia: a meta-analysis of randomized controlled studies with 12-month follow-up. J Clin Gastroenterol. 2014;48:241–7.
29. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, et al. Kyoto global consensus report on *Helicobacter pylori* gastritis. Gut. 2015;64:1353–67.
30. Sobala GM, Crabtree JE, Dixon MF, Schorah CJ, Taylor JD, Rathbone BJ, et al. Acute *Helicobacter pylori* infection: clinical features, local and systemic immune response, gastric mucosal histology, and gastric juice ascorbic acid concentrations. Gut. 1991;32:1415–8.
31. Graham DY, Opekun AR, Osato MS, El-Zimaity HM, Lee CK, Yamaoka Y, et al. Challenge model for *Helicobacter pylori* infection in human volunteers. Gut. 2004;53:1235–43.
32. Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ. Attempt to fulfil Koch's postulates for pyloric *Campylobacter*. Med J Aust. 1985;142:436–9.
33. Morris A, Nicholson G. Ingestion of *Campylobacter pyloridis* causes gastritis and raised fasting gastric pH. Am J Gastroenterol. 1987;82:192–9.
34. Morris AJ, Ali MR, Nicholson GI, Perez-Perez GI, Blaser MJ. Long-term follow-up of voluntary ingestion of *Helicobacter pylori*. Ann Intern Med. 1991;114:662–3.
35. Shah S, Cappell K, Sedgley R, Pelletier C, Jacob R, Bonafede M, et al. Diagnosis and treatment patterns among patients with newly diagnosed *Helicobacter pylori* infection in the United States 2016-2019. Sci Rep. 2023;13:1375.
36. Gong H, Xu HM, Zhang DK. Focusing on *Helicobacter pylori* infection in the elderly. Front Cell Infect Microbiol. 2023;13:1121947.
37. Pilotto A. *Helicobacter pylori*-associated peptic ulcer disease in older patients: current management strategies. Drugs Aging. 2001;18:487–94.
38. Huang Q, Jia X, Chu Y, Zhang X, Ye H. *Helicobacter pylori* infection in geriatric patients: current situation and treatment regimens. Front Med. 2021;8:713908.
39. Collins SM, Denou E, Verdu EF, Bercik P. The putative role of the intestinal microbiota in the irritable bowel syndrome. Dig Liver Dis. 2009;41:850–3.
40. Mach T. The brain-gut axis in irritable bowel syndrome clinical aspects. Med Sci Monit. 2004;10:RA125–31.
41. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci. 2012;13:701–12.
42. Zaki M, Coudron PE, McCuen RW, Harrington L, Chu S, Schubert ML. *H. pylori* acutely inhibits gastric secretion by activating CGRP sensory neurons cou-

pled to stimulation of somatostatin and inhibition of histamine secretion. Am J Physiol Gastrointest Liver Physiol. 2013;304:G715–22.

43. Suzuki H, Matsuzaki J, Hibi T. Ghrelin and oxidative stress in gastrointestinal tract. J Clin Biochem Nutr. 2011;48:122–5.
44. Jun DW, Lee OY, Lee YY, Choi HS, Kim TH, Yoon BC. Correlation between gastrointestinal symptoms and gastric leptin and ghrelin expression in patients with gastritis. Dig Dis Sci. 2007;52:2866–72.
45. Chauhan A, Mullins PA, Taylor G, Petch MC, Schofield PM. Cardioesophageal reflex: a mechanism for “linked angina” in patients with angiographically proven coronary artery disease. J Am Coll Cardiol. 1996;27:1621–8.
46. Wood JD. Neuropathophysiology of functional gastrointestinal disorders. World J Gastroenterol. 2007;13:1313–32.
47. Tougas G, Spaziani R, Hollerbach S, Djuric V, Pang C, Upton AR, et al. Cardiac autonomic function and oesophageal acid sensitivity in patients with noncardiac chest pain. Gut. 2001;49:706–12.
48. Akkaya N, Akkaya S, Polat Y, Turk M, Turk T, Turhal E, et al. *Helicobacter pylori* seropositivity in fibromyalgia syndrome. Clin Rheumatol. 2011;30:43–9.
49. Domínguez-Muñoz JE, Malfertheiner P. Effect of *Helicobacter pylori* infection on gastrointestinal motility, pancreatic secretion and hormone release in asymptomatic humans. Scand J Gastroenterol. 2001;36:1141–7.
50. Neufeld KM, Kang N, Bienenstock J, Foster JA. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol Motil. 2011;23:255–64. e119.
51. Peuhkuri K, Vapaatalo H, Korpela R. Even low-grade inflammation impacts on small intestinal function. World J Gastroenterol. 2010;16:1057–62.
52. Aebischer T, Fischer A, Walduck A, Schlötelburg C, Lindig M, Schreiber S, et al. Vaccination prevents *Helicobacter pylori*-induced alterations of the gastric flora in mice. FEMS Immunol Med Microbiol. 2006;46:221–9.
53. Engstrand L, Lindberg M. *Helicobacter pylori* and the gastric microbiota. Best Pract Res Clin Gastroenterol. 2013;27:39–45.
54. Lagunes-Servin H, Torres J, Maldonado-Bernal C, Pérez-Rodríguez M, Huerta-Yépez S, Madrazo de la Garza A, et al. Toll-like receptors and cytokines are upregulated during *Helicobacter pylori* infection in children. Helicobacter. 2013;18:423–32.
55. Rahimi HR, Rasouli M, Jamshidzadeh A, Farshad S, Firoozi MS, Taghavi AR, et al. New immunological investigations on *Helicobacter pylori*-induced gastric ulcer in patients. Microbiol Immunol. 2013;57:455–62.
56. Romero-Adrián TB, Leal-Montiel J, Monsalve-Castillo F, Mengual-Moreno E, McGregor EG, Perini L, et al. *Helicobacter pylori*: bacterial factors and the role of cytokines in the immune response. Curr Microbiol. 2010;60:143–55.
57. Konturek PC, Brzozowski T, Konturek SJ. Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options. J Physiol Pharmacol. 2011;62:591–9.
58. Ziemssen T, Kern S. Psychoneuroimmunology—cross-talk between the immune and nervous systems. J Neurol 2007;254 Suppl 2:II8–11.
59. Vizi ES, Elenkov IJ. Nonsynaptic noradrenaline release in neuro-immune responses. Acta Biol Hung. 2002;53:229–44.
60. Gwee KA, Teng L, Wong RK, Ho KY, Sutedja DS, Yeoh KG. The response of Asian patients with functional dyspepsia to eradication of *Helicobacter pylori* infection. Eur J Gastroenterol Hepatol. 2009;21:417–24.
61. Ulasoglu C, Isbilen B, Doganay L, Ozen F, Kiziltas S, Tuncer I. Effect of *Helicobacter pylori* eradication on serum ghrelin and obestatin levels. World J Gastroenterol. 2013;19:2388–94.
62. Yang YJ, Sheu BS. Probiotics-containing yogurts suppress *Helicobacter pylori* load and modify immune response and intestinal microbiota in the *Helicobacter pylori*-infected children. Helicobacter. 2012;17:297–304.
63. Yang YJ, Sheu BS, Yang HB, Lu CC, Chuang CC. Eradication of *Helicobacter pylori* increases childhood growth and serum acylated ghrelin levels. World J Gastroenterol. 2012;18:2674–81.
64. Francois F, Roper J, Joseph N, Pei Z, Chhada A, Shak JR, et al. The effect of *H. pylori* eradication on meal-associated changes in plasma ghrelin and leptin. BMC Gastroenterol. 2011;11:37.
65. Deng ZH, Chu B, Xu YZ, Zhang B, Jiang LR. Influence of *Helicobacter pylori* infection on ghrelin levels in children. World J Gastroenterol. 2012;18:5096–100.
66. Tsay FW, Hsu PI. *H. pylori* infection and extra-gastroduodenal diseases. J Biomed Sci. 2018;25:1–8.
67. Marginean CD, Marginean CO, Melit LE. *Helicobacter pylori*-related extraintestinal manifestations—myth or reality. Children. 2022;9:1352.
68. Deng B, Li Y, Zhang Y, Bai L, Yang P. *Helicobacter pylori* infection and lung cancer: a review of an emerging hypothesis. Carcinogenesis. 2013;34:1189–95.
69. Buzás GM. Metabolic consequences of *Helicobacter pylori* infection and eradication. World J Gastroenterol. 2014;20:5226.
70. Chmiela M, Gajewski A, Rudnicka K. *Helicobacter pylori* vs. coronary heart disease-searching for connections. World. J Cardiol. 2015;7:187.
71. Elbehiry A, Marzouk E, Aldubaib M, Abalkhail A, Anagreyyah S, Anajirih N, et al. *Helicobacter pylori* infection: current status and future prospects on diagnostic, therapeutic and control challenges. Antibiotics (Basel). 2023;12:191.

Part V

Disease

Synopsis of *H. pylori*-Associated Diseases

17

Nayoung Kim

Abstract

Helicobacter pylori (*H. pylori*) has urease and *ureI* gene to overcome gastric acid to survive on the acidic gastric mucosa. Colonization with *H. pylori* is a condition that affects the relative risk of developing various clinical disorders of the upper gastrointestinal (GI) tract or extragastroduodenal disorders. That is, long-term inflammation due to *H. pylori* infection causes progressive damage to the gastric mucosa and plays a causative role in a number of important diseases, including duodenal ulcer, gastric ulcer, gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma in addition to atrophic gastritis and intestinal metaplasia. Two decades of intense research into *H. pylori* virulence factors such as the VacA and CagA proteins have revealed many aspects of the relationships between this bacterium, the gastric mucosal surface, and the induction of disease. In addition, *H. pylori* colonization significantly affects the gastric microenvironment, which in turn affects gastric microbiota resulting in possible colonic microbiota changes. Crosstalk between *H. pylori* and GI commensal flora may play a role in *H. pylori*-related carcinogenicity and extragastric manifestations. Disease outcome is the result of the intricate, ongoing interplay between environmental, bacterial, and host factors. In the continuous interactions with the host, the bacteria are able to adapt by mutations and DNA rearrangements, rendering novel genotypes, and overcome the host immune mechanism. On the host side, variations in the host immune response to the chronic presence of *H. pylori* and genetic polymorphism of host directly impact *H. pylori*-associated gastric disease, resulting in the disease outcome. However, *H. pylori* colonization pattern such as antrum-predominant gastritis and corpus-predominant pangastritis is known to determine the disease outcome, and this gastritis pattern is mainly decided by the acid secretion when *H. pylori* starts to colonize in the gastric mucosa.

Keywords

Synopsis · Disease · Outcome · *Helicobacter pylori*

17.1 Introduction

Colonization with *Helicobacter pylori* (*H. pylori*) is not a disease in itself but a condition that affects the relative risk of developing various clinical

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disorders of the upper gastrointestinal (GI) tract or extragastroduodenal disorders [1]. *H. pylori* causes progressive damage to the gastric mucosa and is now accepted as playing a causative role in a number of important diseases, including duodenal ulcer (DU), gastric ulcer, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [2–4]. Indeed, *H. pylori*-induced gastritis is considered as the most important risk factor for peptic ulcer and its complications as well as for gastric cancer (GC) [4]. Testing for *H. pylori* therefore has no relevance by itself but should be performed to find the cause of an underlying condition, such as peptic ulcer disease, or for the purpose of disease prevention, such as in GC relatives [5, 6]. In these cases, a positive test result justifies treatment, and a negative test result may indicate the need to search for other etiologic factors or preventive measures [1]. For these reasons, a correct understanding of the clinical course of *H. pylori*-associated disorders and the effect of *H. pylori* eradication is needed. Two decades of intense research into *H. pylori* virulence factors such as the VacA and CagA proteins have revealed many aspects of the relationships between this bacterium, the gastric mucosal surface, and the induction of disease [1]. The GI microbiota and the host generate a complex network of interactions that transcends the boundaries of the GI tract, forging intimate connections with all aspects of human physiology, including metabolic, immune, and neuroendocrine systems [7]. The crosstalk has been known to be mediated by microbial-derived biochemical signals that are absorbed into the blood and circulated throughout the human body; by signals relayed by the enteric nervous system that transmit microbiota-derived cues to the central nervous system; and by immune cells that perceive local microbial signals in the GI tract and are trafficked throughout the body [8–10]. For instance until now, the reason for the increment of GC possibility was explained by atrophic gastritis (AG), intestinal metaplasia (IM), dysplasia, and GC cascade derived from *H. pylori* infection. However, recent research discovered that other more complicate factors including gastric microbiota and immune mechanisms contribute to GC cascade. Strain-to-strain genetic variability in bacterial virulence factors such as *vacA* and *cagA* not only affects the ability of the organism to colonize and cause disease but also affects inflammation and gastric acid output [1]. The decrease gastric output due to *H. pylori*-associated gastritis can explain why the prevalence of reflux gastritis decreases in the *H. pylori*-infected host. Long-term interaction between *H. pylori* and host causes fundamental change to both of *H. pylori* and host. That is, in the continuous interactions with the host, the bacteria are able to adapt to the host condition by mutations and DNA rearrangements. On the host side, variations in the host immune response to the chronic presence of *H. pylori* directly impact *H. pylori*-associated gastric disease and affect gastric acid output and thereby the density and location of *H. pylori*. The change of ecosystem of stomach also looks like to affect the pattern of esophageal and colonic diseases. In this chapter, synopsis of *H. pylori*-associated diseases such as gastritis, peptic ulcer, nonulcer dyspepsia, MALT lymphoma, GC, and extragastric disorders will be briefly described.

17.2 *H. pylori*-Associated Gastric Diseases

Although gastric colonization with *H. pylori* inevitably induces histologic gastritis in the infected individuals, 10–20% of *H. pylori*-positive patients have risk of developing ulcer disease and a 1–2% risk of developing noncardiac GC [11–13]. The risk of development of these disorders in the presence of *H. pylori* infection depends on a variety of bacterial, host, and environmental factors that mostly relate to the pattern and severity of gastritis [1] (Fig. 17.1).

17.2.1 Acute and Chronic Gastritis

Chronic active gastritis, which occurs after colonization with *H. pylori*, can be observed in all *H. pylori*-positive subjects. The intragastric distribution and severity of this chronic inflamma-

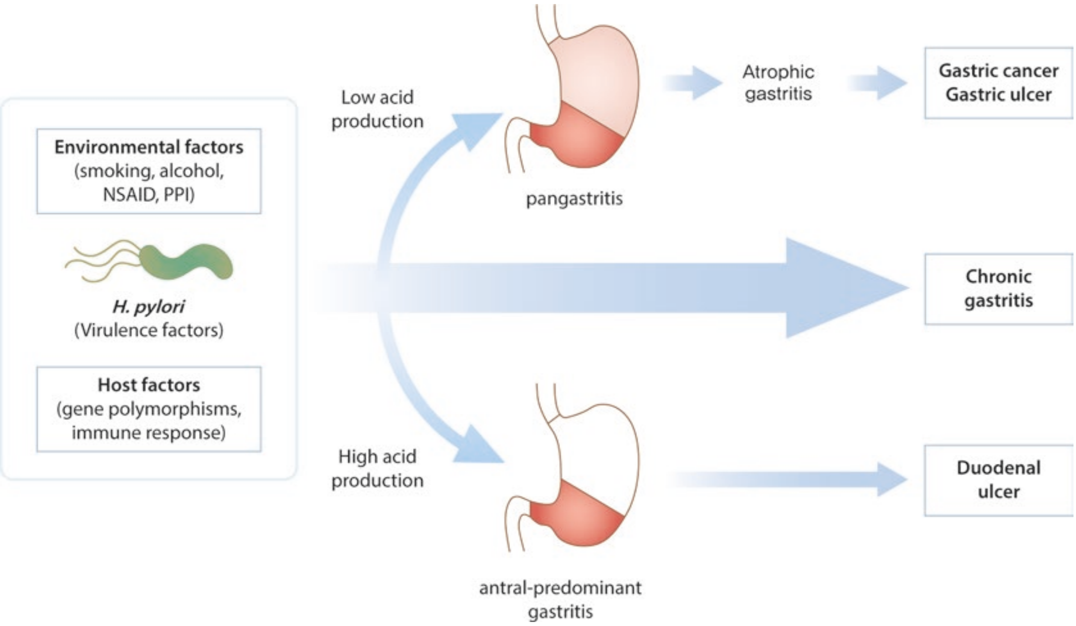
Environmental factors
(smoking, alcohol, NSAID, PPI)
H. pylori
(Virulence factors)
Host factors
(gene polymorphisms, immune response)
Low acid production
pangastritis
Atrophic gastritis
Gastric cancer
Gastric ulcer
Chronic gastritis
High acid production
antral-predominant gastritis
Duodenal ulcer

Fig. 17.1 Schematic representation of the factors contributing to gastric pathology and disease outcome in *H. pylori* infection. *NSAID* Nonsteroidal anti-inflammatory drug, *PPI* Proton pump inhibitor (Modified from Kusters et al. [1])

tory process depend on a variety of factors, such as characteristics of the colonizing strain, host genetics and immune response, diet, and the level of acid production [1]. *H. pylori*-induced ulcer disease, GC, and MALT lymphoma are all complications of this chronic inflammation; ulcer disease and GC in particular occur in those individuals and at those sites with the most severe inflammation [1]. Colonization with *H. pylori* virtually always leads to infiltration of the gastric mucosa in both the antrum and corpus with neutrophils and mononuclear cells, which consist of chronic inflammatory process.

17.2.1.1 Acute Gastritis

When *H. pylori* enters the stomach of a human host and colonizes itself on gastric mucosa, heavy neutrophils infiltrate into the mucosa. Neutrophil infiltration and the severity of gastric mucosa damage are closely related, and it seems like gastric epithelial cell damage is caused by infiltrated leukocytes due to *H. pylori* infection [14]. Once the bacteria enter the stomach and induce infection, subjects developed variable GI and constitutional symptoms. Dyspeptic symptoms were mild to moderate in severity and typically appeared during the first week after challenge and peaked between days 9 and 12 [15]. These symptoms start within the first week of infection, and these symptoms reach their maximum degrees on 9–12 days after the infection [15]. Then, the symptoms are attenuated by themselves, and most of the symptoms are gone after 2 weeks of the infection [15–18]. It is unclear whether this initial colonization can be followed by spontaneous clearance and resolution of gastritis and, if so, how often this occurs [1]. Follow-up studies of young children with serology or breath tests suggested that infection may spontaneously disappear in some patients in this age group [19–21]. However, it does not occur in adults except disappearance of *H. pylori* in case of AG and IM [22]. In addition, studies of homozygotic twins showed a concordance in their *H. pylori* status irrespective of whether they had grown up together or apart, but this concordance was not observed among heterozygotic twins [23]. These results suggest that some individuals are prone to *H. pylori* colonization, while others may be able to prevent colonization or clear an established infection [1].

17.2.1.2 Chronic Gastritis

When colonization does become persistent, a close correlation exists between the level of acid secretion and the distribution of gastritis, which determines the gastric hormonal responses with dynamic changes of gastric mucosa. Finally, they determine the outcomes of *H. pylori* infection. In subjects with intact acid secretion, *H. pylori* in particular colonizes the gastric antrum, where few acid secretory parietal cells are present because they like weak acid environment such as pH 5.0 instead of strong acid. This colonization pattern is associated with an antrum-predominant gastritis [1] (Fig. 17.1). Histological evaluation of gastric corpus specimens in these cases reveals limited chronic inactive inflammation and low numbers of superficially colonizing *H. pylori* bacteria [1]. In this state DU can easily develop [1] (Fig. 17.1). Subjects in whom acid secretion is impaired have a more even distribution of bacteria in the antrum and corpus, and bacteria in the corpus are in closer contact with the mucosa, leading to a corpus-predominant pangastritis [1, 24] (Fig. 17.1). In this state, the acid secretion will decrease and could be a condition for gastric ulcer or GC development when the bacteria continuously interact with the host. As predisposition condition of DU and GC is contradictory, the chance of development of GC is known to be half in case of DU history. Similarly, there was a report that GC occurred only in patients with previous benign gastric ulcer (BGU) and not in patients with former DU [25]. When this chronic inflammation continues, AG defined as the loss of glands [26] occurs and slowly progresses to IM, which is defined as the replacement of the surface, foveolar, and glandular epithelium in the gastric mucosa by intestinal epithelium with the presence of Paneth cells, goblet cells, and absorptive cells [27]. *H. pylori* infection was the most important risk factor of AG and IM, and AG is considered to be an antecedent to IM [28]. Actually the time difference of AG and IM could be different, but Korean report suggested 10 years [28]. According to the Correa model, the pathogenesis of intestinal-type GC can be explained by a multistep process from chronic gastritis through AG, IM, and dysplasia to cancer [26]. The risk of GC increases with greater extent and higher degree of gastric mucosal atrophy [27] and more than tenfold by IM [29]. Several studies suggest that AG and IM are not related with gastroduodenal symptoms [30], but they are major precursor lesions of GC [29, 31, 32].

17.2.2 Nonulcer Dyspepsia

Nonulcer or functional dyspepsia (FD) is defined as the presence of symptoms of upper GI distress without any identifiable structural abnormality during diagnostic workup, in particular including upper GI endoscopy [33]. Uninvestigated dyspepsia is defined as the presence of dyspeptic symptoms for which no further diagnostic evaluation has been performed. These symptoms are frequently experienced by 20–30% of the adult population of the Western world [33]. FD was divided into four symptoms based on the Rome III criteria as follows: epigastric pain, epigastric burning, bothersome postprandial fullness, and early satiation (prevents finishing regular-sized meals). Rome III criteria classified FD into two subcategories of FD based on cohort and population-based studies: postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS) [33]. A recent meta-analysis reported that the odds ratio (OR) for the occurrence of postinfectious FD was 2.54 (95% confidence interval [CI], 1.76–3.65) more than 6 months after acute gastroenteritis compared with the control [34]. Another meta-analysis found an OR of 2.18 (95% CI, 1.70–2.81) for FD risk following acute gastroenteritis [35]. These results suggest that GI infection is associated with an increased risk of FD, supporting an inflammatory and immunological mechanism in the pathogenesis of FD [36]. *H. pylori* infection is the main cause of gastroduodenal inflammation [37] and provokes activation of a complex cytokine and chemokine response in the gastric mucosa [38], which may induce dyspepsia [39]. However, 30–60% of patients with functional dyspepsia carry *H.*

pylori, but this prevalence is not much different from that in the unaffected population [32, 40]. This result could be explained by that FD is a multifactorial disease rather than *H. pylori*-dependent disease. Thus, the effect of eradication on FD could be the only method whether *H. pylori* infection is related with FD or not. A Cochrane meta-analysis was performed on 17 randomized controlled trials and identified an association between *H. pylori* eradication and improvement in FD symptoms [41]. A small but significant benefit of *H. pylori* eradication therapy was observed with a number needed to treat of 14 (95% CI, 10–25) [41]. In another recent study, the number needed to treat was 8 [42]. In addition, a systemic review and meta-analysis from China reported that the summary OR for improvement in FD patients after *H. pylori,* eradication was 3.61 (95% CI, 2.62–4.98) [43]. Similarly, a recent meta-analysis of randomized controlled trials with 12 months follow-up revealed that dyspeptic symptoms were significantly improved in the eradication group (OR 1.38; 95% CI, 1.18–1.62) [44]. Based on these considerations, the Kyoto global consensus proposed that patients who remain symptomatic after successful *H. pylori* eradication should be regarded as having *H. pylori*-associated dyspepsia separated from FD [45]. In addition, *H. pylori* eradication has been recommended as first-line treatment for dyspeptic patients with *H. pylori* infection, because eradication therapy for dyspeptic symptoms is better than placebo in *H. pylori*-infected dyspeptic patients [45]. However, as symptom resolution takes months after completion of eradication therapy, this time delay makes some scholars to not agree with this proposal.

17.2.3 Gastric or Duodenal Ulcers

Gastric or duodenal ulcers are defined as mucosal defects with a diameter of at least 0.5 cm penetrating through the muscularis mucosa [1], but the guideline could be different to 0.3 cm. BGU mostly occurs along the lesser curvature of the stomach, in particular, at the transition from the corpus to antrum mucosa [46]. DU usually occurs in the duodenal bulb, which is the area most exposed to gastric acid. In Western countries, DU is approximately fourfold more common than BGU; elsewhere, BGU is more common, especially, when aged population increases [1]. For instance, the ratio of BGU ($n = 265$) to DU ($n = 210$) was 1.26:1 in Korea [47]. In terms of age, DU frequently occurs between 20 and 50 years of age, while BGU predominantly arises in subjects over 40 years old. Recently *H. pylori* prevalence rapidly decreases, the mean age of DU also increases, but still there is age difference between DU and BGU [47]. Both gastric and duodenal ulcer diseases are strongly related to *H. pylori*. In the first decade after the discovery of *H. pylori*, approximately 95% of DU and 85% of BGU occurred in the presence of *H. pylori* infection [13]. Several cohort studies estimated that the lifetime risk for ulcer disease in *H. pylori*-positive subjects is three to ten times higher than in *H. pylori*-negative subjects [48]. Furthermore, *H. pylori* eradication provided strong evidence for a causal relationship between *H. pylori* and ulcer disease by showing that eradication of this bacterium strongly reduced the risk of recurrent ulcer disease [49–54]. Ulcer development in the presence of *H. pylori* is influenced by a variety of host and bacterial factors [55]. If acid output is decreased, the gastric transitional zone between the corpus and antrum is the place where gastric ulcer can occur [1]. If acid production is normal to high, the most severe inflammation usually is found in the distal stomach and proximal duodenum, giving rise to juxtapyloric and duodenal ulcer disease [1]. Recently the incidence and prevalence of peptic ulcer have been decreasing [56]. Hygiene improvement and low prevalence of *H. pylori* infection are suspected as main reasons, but the increase of aging population and the use of nonsteroidal anti-inflammatory drug (NSAID) become the major cause of peptic ulcer diseases instead of *H. pylori* infection. In addition, idiopathic peptic ulcer rapidly increases [57].

17.2.4 Gastric MALT Lymphoma

The gastric mucosa does not normally contain lymphoid tissue, but MALT nearly always appears in response to colonization with *H. pylori*. In rare cases, a monoclonal population of B cells may arise from this tissue and slowly proliferate to form a MALT lymphoma [1]. The histological criteria for the diagnosis of gastric MALT lymphoma and the differentiation from polyclonal reactive infiltrates remain controversial. In particular, diagnosis is based on histological appearance during routine microscopy and on demonstration of clonality by immunohistochemistry or molecular techniques, such as PCR. Nearly all MALT lymphoma patients are *H. pylori* positive [58], and *H. pylori*-positive subjects have a significantly increased risk for the development of gastric MALT lymphoma [59]. Because of the diagnostic controversies and the relative rarity of this disorder, the exact incidence in *H. pylori*-positive subjects is unknown, but MALT lymphomas occur in less than 1% of *H. pylori*-positive subjects [60]. *H. pylori* eradication can lead to complete remission in approximately 60–80% of patients with stage IE MALT lymphoma confined to the stomach, but some 10% continue to have signs of minimal residual disease, and the remainder shows no response or disease progression [61–65]. Ten to 35% of those who initially reach complete remission after *H. pylori* eradication show recurrent disease during further follow-up. A major predictor for response appears to be the presence of a t(11;18)(q21;q21) translocation. This translocation is associated with *API2-MALT1* fusion, the former being involved in regulation of apoptosis, the latter resembling a caspase-like protein, but with as-yet-unknown biological function. Together, the fusion leads to suppression of apoptosis. Several studies have reported that MALT lymphomas with this translocation do not at all or only rarely respond to *H. pylori* eradication [65–67].

17.2.5 Gastric Cancer

Despite the global decrease, GC is still a burdensome disease internationally. GC is currently the fifth most common cancer, with approximately 1 million new cases diagnosed worldwide each year [68], and ranks as the fourth leading cause of cancer-related death [69]. The prognosis of GC patients depends on the tumor stage at the time of initial diagnosis that the 5-year survival rates of early GC exceeding 90% make a striking contrast to those of advanced GC reaching below 50% [70–73]. Therefore, the efforts to detect GC when the tumor is in early stage can reduce the overall socioeconomic burden of GC. Traditionally, gastric cancer was thought to develop due to dietary carcinogens produced by old ways of food preservation such as smoking and salting. However, in recent decades, *H. pylori* has been marked as the main culprit of GC development [74]. According to the Lauren’s classification system [75], GC can be classified into two major histological variants: an intestinal-type and a diffuse-type. It is generally accepted that intestinal-type gastric adenocarcinoma arises through a multistep process from chronic gastritis that progresses through stages of atrophy, IM, and dysplasia and finally intestinal-type cancer [26]. On the other hand, diffuse-type GC is thought to be primarily genetically determined and to be less associated with environmental factors than the intestinal type and not to progress through severe AG [76]. In a meta-analysis of 19 cohort or case-control studies, a summary odds ratio for GC was estimated to be 1.92 (95% CI, 1.32–2.78) in *H. pylori*-infected subjects compared to uninfected subjects regardless of histological type of GC such as intestinal-type or diffuse-type [77]. Furthermore, several randomized control studies have shown that *H. pylori* eradication reduces the risk of GC [78]. Among those, the most recent study reported that *H. pylori* eradication reduced GC incidence by 25% [79], confirming the importance of *H. pylori* eradication for the prevention of GC. In addition to *H. pylori* infection, low socioeconomic status, smoking, and family history of GC were established as risk factors of GC [5, 6, 80–84]. Factors such as alcohol, fruits, vegetables, and salty and spicy food intake have been subjects of controversy [84–87]. Regarding ABO blood type's association with GC, blood group A was associated with increased risk of GC [88, 89], and B allele was associated with decreased risk of GC in a Japanese [90] and Korean study [91]. These findings suggest that GC is a multifactorial disease with *H. pylori* being the primary cause, and *H. pylori*'s effect on carcinogenesis is modulated by microbial, environmental, and host factors [92].

17.3 Gastroesophageal Reflux Disease, Barrett's Esophagus, and Esophageal Adenocarcinorma

Chronic gastric acid exposure or duodenal bile in the distal esophagus is considered to be the primary factor in the pathogenesis of reflux esophagitis. In addition, it became widely accepted that reflux may cause chronic esophageal injury and promote carcinogenesis in Barrett's esophagus. Many studies suggested that *H. pylori* might protect against the development of gastroesophageal reflux disease (GERD) and as such also be of benefit to their hosts [1]. This concept came from repeated observations of a low prevalence of *H. pylori* among GERD patients, particularly of more virulent strains [93], opposing time, and geographical trends for *H. pylori* prevalence compared with the incidence of GERD and its complications, a potentially increased incidence of GERD after *H. pylori* eradication [94], and the recognition that *H. pylori*-induced corpus gastritis reduced acid secretion. The hypothesis was that *H. pylori*-induced inflammation of the gastric corpus had an acid-suppressive effect, thus preventing patients from contracting GERD. Similarly, the incidences of Barrett's esophagus and esophageal adenocarcinorma are inversely associated with *H. pylori* infection prevalence [95, 96]. Interestingly, the composition of core esophageal microbiota in healthy controls was reported to be different at the phylum and genus levels from patients with reflux esophagitis or Barrett's esophagus suggesting that the altered bacterial microenvironment may contribute substantially to esophageal mucosa injury and further carcinogenesis [7]. The *H. pylori*-positive stomach produces less acid which causes alteration of the microbial community in the distal esophagus. However, there was no evidence that *H. pylori* eradication has a considerable impact on either the new development of GERD [97, 98]. These data show that although epidemiologic data suggest that there may be an inverse relation between *H. pylori* and GERD, Barrett's esophagus and esophageal adenocarcinorma, the risk for new development or worsening of preexistent GERD or its complication is not an issue in the decision of whether or not to treat *H. pylori*.

17.4 Extraintestinal Manifestations of *H. pylori* Infection

H. pylori has been linked to a variety of extraintestinal disorders. These include coronary heart disease (CHD), asthma, dermatological disorders such as rosacea and idiopathic urticaria, autoimmune thyroid disease and idiopathic thrombocytopenic purpura, iron deficiency anemia, Raynaud's phenomenon, scleroderma, migraine, and neurologic disorders such as Guillain-Barré syndrome [1]. The underlying hypothetical mechanisms include chronic low-grade activation of the coagulation cascade, accelerating atherosclerosis, and antigenic mimicry between *H. pylori* and host epitopes leading to autoimmune disorders [99]. In addition, metabolic syndrome, a constellation of metabolic disturbances, including hypertension, hyperglycemia, dyslipidemia, and central obesity has been reported to have a positive association with *H. pylori* that has been reported worldwide [100]. Furthermore, there was a positive correlation between *H. pylori* and CHD [101–104] or ischemic stroke [105], especially in the presence of CagA. Several groups in particular have studied patients with idiopathic thrombocytopenic purpura and showed that when these patients are colonized with *H. pylori*, eradication therapy has a significant effect over placebo for improvement of thrombocyte counts [106–108] and iron deficiency anemia [109, 110]. Our team also reported that *H. pylori* eradication prevented CHD, and its effects differed depending on age and sex [111]. That is, *H. pylori* eradication prevented CHD in younger males (≤ 65 years) and older females (>65 years) [111]. Currently, according to American College of Gastroenterology Guideline and the Maastricht VI/Florence Consensus Report, *H. pylori* eradication is recommended in unexplained iron deficiency anemia and idiopathic thrombocytopenic purpura so far [4, 112].

17.5 Conclusions

H. pylori causes progressive damage to the gastric mucosa and is now accepted as playing a causative role in a number of important diseases, including gastritis, DU, gastric ulcer, GC gastric MALT lymphoma, and extragastric disorders. This disease outcome is the result of the intricate, ongoing interplay between environmental, bacterial, and host factors. The GI tract is a complex and dynamic ecosystem with interplay between various gut mucosal cells and their defense molecules, the immune system, food particles, and resident microbes. *H. pylori* infection reduces gastric acid and changes the gastric microenvironment, which in turn causes subsequent GI commensal microbiota colonization. Long-term interaction between *H. pylori* and host causes fundamental change to both *H. pylori* and host. That is, in the continuous interactions with the host, the bacteria are able to adapt to the host condition by mutations and DNA rearrangements. On the host side, variations in the host immune response to the chronic presence of *H. pylori* directly impact *H. pylori*-associated gastric disease outcome and affect gastric acid output. It is well known that *H. pylori* colonization pattern such as antrum-predominant gastritis and corpus-predominant pangastritis is mainly decided by the acid secretion when *H. pylori* starts to colonize in the gastric mucosa because *H. pylori* likes the weak acid condition instead of strong acid. Nowadays scientific efforts have been focused on the benefits of treating and eradicating *H. pylori*, which will inevitably cause changes of gut-microbial–host-immune/metabolic axis.

References

1. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. Clin Microbiol Rev. 2006;19:449–90.
2. Malfertheiner P, Chan FK, McColl KE. Peptic ulcer disease. Lancet. 2009;374:1449–61.
3. Fock KM, Graham DY, Malfertheiner P. *Helicobacter pylori* research: historical insights and future directions. Nat Rev Gastroenterol Hepatol. 2013;10:495–500.
4. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection—the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
5. Shin CM, Kim N, Yang HJ, Cho SI, Lee HS, Kim JS, et al. Stomach cancer risk in gastric cancer relatives: interaction between *H. pylori* infection and family history of gastric cancer for the risk of stomach cancer. J Clin Gastroenterol. 2010;44:e34–9.
6. Shin CM, Kim N, Lee HS, Lee DH, Kim JS, Jung HC, et al. Intrafamilial aggregation of gastric cancer: a comprehensive approach including environmental factors, *Helicobacter pylori* virulence and genetic susceptibility. Eur J Gastroenterol Hepatol. 2011;23:411–7.
7. Chen CC, Liou JM, Lee YC, Hong TC, El- Omar EM, Wu MS. The interplay between *Helicobacter pylori* and gastrointestinal microbiota. Gut Microbes. 2021;13:1.
8. Donia MS, Fischbach MA. Small molecules from the human microbiota. Science. 2015;349:1254766.
9. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci. 2012;13:701–12.
10. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science. 2012;336:1268–73.
11. Ernst PB, Gold BD. The disease spectrum of *Helicobacter pylori*: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. Annu Rev Microbiol. 2000;54:615–40.
12. Kuipers EJ. Review article: exploring the link between *Helicobacter pylori* and gastric cancer. Aliment Pharmacol Ther. 1999;13 (Suppl 1):3–11.
13. Kuipers EJ, Thijs JC, Festen HP. The prevalence of *Helicobacter pylori* in peptic ulcer disease. Aliment Pharmacol Ther. 1995;9 (Suppl 2):59–69.
14. Dundon WG, Nishioka H, Polenghi A, Papinutto E, Zanotti G, Montemurro P, et al. The neutrophil-

activating protein of *Helicobacter pylori*. Int J Med Microbiol. 2002;291:545–50.

15. Graham DY, Opekun AR, Osato MS, El-Zimaity HM, Lee CK, Yamaoka Y, et al. Challenge model for *Helicobacter pylori* infection in human volunteers. Gut. 2004;53:1235–43.
16. Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ. Attempt to fulfill Koch's postulates for pyloric *Campylobacter*. Med J Aust. 1985;142:436–9.
17. Morris A, Nicholson G. Ingestion of *Campylobacter pyloridis* causes gastritis and raised fasting gastric pH. Am J Gastroenterol. 1987;82:192–9.
18. Morris AJ, Ali MR, Nicholson GI, Perez-Perez GI, Blaser MJ. Long-term follow-up of voluntary ingestion of *Helicobacter pylori*. Ann Intern Med. 1991;114:662–3.
19. Granstrom M, Tindberg Y, Blennow M. Seroepidemiology of *Helicobacter pylori* infection in a cohort of children monitored from 6 months to 11 years of age. J Clin Microbiol. 1997;35:468–70.
20. Malaty HM, Graham DY, Wattigney WA, Srinivasan SR, Osato M, Berenson GS. Natural history of *Helicobacter pylori* infection in childhood: 12-year follow-up cohort study in a biracial community. Clin Infect Dis. 1999;28:279–82.
21. Pérez-Pérez GI, Sack RB, Reid R, Santosham M, Croll J, Blaser MJ. Transient and persistent *Helicobacter pylori* colonization in native American children. J Clin Microbiol. 2003;41:2401–7.
22. Kang HY, Kim N, Park YS, Hwang JH, Kim JW, Jeong SH, et al. Progression of atrophic gastritis and intestinal metaplasia driving *Helicobacter pylori* out of the gastric mucosa. Dig Dis Sci. 2006;51:2310–5.
23. Malaty HM, Engstrand L, Pedersen NL, Graham DY. *Helicobacter pylori* infection: genetic and environmental influences. A study of twins. Ann Intern Med. 1994;120:982–6.
24. Kuipers EJ, Uyterlinde AM, Peña AS, Hazenberg HJ, Bloemena E, Lindeman J, et al. Increase of *Helicobacter pylori*-associated corpus gastritis during acid suppressive therapy: implications for long-term safety. Am J Gastroenterol. 1995;90:1401–6.
25. Take S, Mizuno M, Ishiki K, Nagahara Y, Yoshida T, Yokota K, et al. The effect of eradicating *Helicobacter pylori* on the development of gastric cancer in patients with peptic ulcer disease. Am J Gastroenterol. 2005;100:1037–42.
26. Correa P. Chronic gastritis: a clinico-pathological classification. Am J Gastroenterol. 1988;83:504–9.
27. de Vries AC, Kuipers EJ. Epidemiology of premalignant gastric lesions: implications for the development of screening and surveillance strategies. Helicobacter. 2007;12 (Suppl 2):22–31.
28. Kim N, Park YS, Cho SI, Lee HS, Choe G, Kim IW, et al. Prevalence and risk factors of atrophic gastritis and intestinal metaplasia in a Korean population without significant gastroduodenal disease. Helicobacter. 2008;13:245–55.
29. Leung WK, Sung JJ. Review article: intestinal metaplasia and gastric carcinogenesis. Aliment Pharmacol Ther. 2002;16:1209–16.
30. Kim SE, Park HK, Kim N, Joo YE, Baik GH, Shin JE, et al. Prevalence and risk factors of functional dyspepsia: a nationwide multicenter prospective study in Korea. J Clin Gastroenterol. 2014;48:e12–8.
31. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, et al. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med. 2001;345:784–9.
32. Ohata H, Kitauchi S, Yoshimura N, Mugitani K, Iwane M, Nakamura H, et al. Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer. Int J Cancer. 2004;109:138–43.
33. Tack J, Talley NJ, Camilleri M, Holtmann G, Hu P, Malagelada JR, et al. Functional gastroduodenal disorders. Gastroenterology. 2006;130:1466–79.
34. Futagami S, Itoh T, Sakamoto C. Systematic review with meta-analysis: post-infectious functional dyspepsia. Aliment Pharmacol Ther. 2015;41:177–88.
35. Pike BL, Porter CK, Sorrell TJ, Riddle MS. Acute gastroenteritis and the risk of functional dyspepsia: a systematic review and meta-analysis. Am J Gastroenterol. 2013;108:1558–63.
36. Gwee KA. Post-infectious irritable bowel syndrome, an inflammation-immunological model with relevance for other IBS and functional dyspepsia. J Neurogastroenterol Motil. 2010;16:30–4.
37. Suzuki H, Hibi T, Marshall BJ. *Helicobacter pylori*: present status and future prospects in Japan. J Gastroenterol. 2007;42:1–15.
38. D'Elios MM, Andersen LP. Inflammation, immunity, and vaccines for *Helicobacter pylori*. Helicobacter. 2009;14 (Suppl 1):21–8.
39. Suzuki H, Matsuzaki J, Hibi T. What is the difference between *Helicobacter pylori*-associated dyspepsia and functional dyspepsia? J Neurogastroenterol Motil. 2011;17:124–30.
40. Talley NJ, Hunt RH. What role does *Helicobacter pylori* play in dyspepsia and nonulcer dyspepsia? Arguments for and against *H. pylori* being associated with dyspeptic symptoms. Gastroenterology. 1997;113:S67–77.
41. Moayyedi P, Soo S, Deeks J, Delaney B, Harris A, Innes M, et al. Eradication of *Helicobacter pylori* for non-ulcer dyspepsia. Cochrane Database Syst Rev. 2006;(2):CD002096.
42. Mazzoleni LE, Sander GB, Francesconi CF, Mazzoleni F, Uchoa DM, De Bona LR, et al. *Helicobacter pylori* eradication in functional dyspepsia: HEROES trial. Arch Intern Med. 2011;171:1929–36.
43. Jin X, Li YM. Systematic review and meta-analysis from Chinese literature: the association between *Helicobacter pylori* eradication and improvement of functional dyspepsia. Helicobacter. 2007;12:541–6.
44. Zhao B, Zhao J, Cheng WF, Shi WJ, Liu W, Pan XL, et al. Efficacy of *Helicobacter pylori* eradication

therapy on functional dyspepsia: a meta-analysis of randomized controlled studies with 12-month follow-up. J Clin Gastroenterol. 2014;48:241–7.

45. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, et al. Kyoto global consensus report on *Helicobacter pylori* gastritis. Gut. 2015;64:1353–67.
46. Van Zanten SJ, Dixon MF, Lee A. The gastric transitional zones: neglected links between gastroduodenal pathology and *Helicobacter* ecology. Gastroenterology. 1999;116:1217–29.
47. Kim JJ, Kim N, Lee BH, Kang JM, Seo P, Lim MK, et al. Risk factors for development and recurrence of peptic ulcer disease. Korean J Gastroenterol. 2010;56:220–8.
48. Nomura A, Stemmermann GN, Chyou PH, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and the risk for duodenal and gastric ulceration. Ann Intern Med. 1994;120:977–81.
49. Rauws EA, Tytgat GN. Cure of duodenal ulcer associated with eradication of *Helicobacter pylori*. Lancet. 1990;335:1233–5.
50. Kim N, Lim SH, Lee KH, Choi SE. Long-term effect of *Helicobacter pylori* eradication on gastric metaplasia in patients with duodenal ulcer. J Clin Gstroenterol. 1998;27:246–52.
51. Kim N, Oh JH, Lee CG, Lim C, Won KH, Choi WR, et al. Effect of *H. pylori* eradication on the benign gastric ulcer recurrence—a 24 month follow-up study. Korean J Intern Med. 1999;14:9–14.
52. Hentschell E, Brandstätter G, Dragosics B, Hirschl AM, Nemec H, Schütze K, et al. Effect of ranitidine and amoxicillin plus metronidazole on the eradication of *Helicobacter pylori* and the recurrence of duodenal ulcer. N Engl J Med. 1993;328:308–12.
53. Treiber G, Lambert JR. The impact of *Helicobacter pylori* eradication on peptic ulcer healing. Am J Gastroenterol. 1998;93:1080–4.
54. van der Hulst RW, Rauws EA, Köycü B, Keller JJ, ten Kate FJ, Dankert J, et al. *Helicobacter pylori* reinfection is virtually absent after successful eradication. J Infect Dis. 1997;176:196–200.
55. Kim JY, Kim N, Nam RH, Suh JH, Chang H, Lee JW, et al. The association of polymorphisms in virulence factor of *Helicobacter pylori* and gastroduodenal diseases in South Korea. J Gastroenterol Hepatol. 2014;29:984–91.
56. Sung JJ, Kuipers EJ, El-Serag HB. Systematic review: the global incidence and prevalence of peptic ulcer disease. Aliment Pharmacol Ther. 2009;29:938–46.
57. Kim JJ, Kim N, Park HK, Jo HJ, Shin CM, Lee SH, et al. Clinical characteristics of patients diagnosed as peptic ulcer disease in the third referral center in 2007. Korean J Gastroenterol. 2012;59:338–46.
58. Eidt S, Stolte M, Fischer R. *Helicobacter pylori* gastritis and primary gastric non-Hodgkin's lymphomas. J Clin Pathol. 1994;47:436–9.
59. Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, et al. *Helicobacter pylori* infection and gastric lymphoma. N Engl J Med. 1994;330:1267–71.
60. Berg DE, Hoffman PS, Appelmelk BJ, Kusters JG. The *Helicobacter pylori* genome sequence: genetic factors for long life in the gastric mucosa. Trends Microbiol. 1997;5:468–74.
61. de Mascarel A, Ruskone-Fourmestraux A, Lavergne-Slove A, Megraud F, Dubus P, Merlio JP. Clinical, histological and molecular follow-up of 60 patients with gastric marginal zone lymphoma of mucosa-associated lymphoid tissue. Virchows Arch. 2005;446:219–24.
62. Fischbach W, Goebeler-Kolve ME, Dragosics B, Greiner A, Stolte M. Long term outcome of patients with gastric marginal zone B cell lymphoma of mucosa associated lymphoid tissue (MALT) following exclusive *Helicobacter pylori* eradication therapy: experience from a large prospective series. Gut. 2004;53:34–7.
63. Nakamura S, Matsumoto T, Suekane H, Nakamura S, Matsumoto H, Esaki M, et al. Long-term clinical outcome of *Helicobacter pylori* eradication for gastric mucosa-associated lymphoid tissue lymphoma with a reference to second-line treatment. Cancer. 2005;104:532–40.
64. Wundisch T, Thiede C, Morgner A, Dempfle A, Günther A, Liu H, et al. Long-term follow-up of gastric MALT lymphoma after *Helicobacter pylori* eradication. J Clin Oncol. 2005;23:8018–24.
65. Choi YJ, Kim N, Paik JH, Kim JM, Lee SH, Park YS, et al. Characteristics of *Helicobacter pylori*-positive and *Helicobacter pylori*-negative gastric mucosa-associated lymphoid tissue lymphoma and their influence on clinical outcome. Helicobacter. 2013;18:197–205.
66. Inagaki H, Nakamura T, Li C, Sugiyama T, Asaka M, Kodaira J, et al. Gastric MALT lymphomas are divided into three groups based on responsiveness to *Helicobacter pylori* eradication and detection of API2-MALT1 fusion. Am J Surg Pathol. 2004;28:1560–7.
67. Liu H, Ye H, Ruskone-Fourmestraux A, De Jong D, Pileri S, Thiede C, et al. T(11;18) is a marker for all stage gastric MALT lymphomas that will not respond to *H. pylori* eradication. Gastroenterology. 2002;122:1286–94.
68. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136:E359–86.
69. Statistics Korea. Causes of death statistics in 2020. http://kostat.go.kr/portal/eng/pressReleases/1/index.board?bmode=read&bSeq=&aSeq=414516&pageNo=5&rowNum=10&navCount=10&currPg=&searchInfo=&sTarget=title&sTxt=deat. Accessed 19 Apr 2023.
70. Everett SM, Axon AT. Early gastric cancer in Europe Gut. 1997;41:142–50.
71. Leung WK, Wu MS, Kakugawa Y, Kim JJ, Yeoh KG, Goh KL, et al. Screening for gastric cancer in Asia: current evidence and practice. Lancet Oncol. 2008;9:279–87.

72. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet. 2010;376:687–97.
73. Lello E, Furnes B, Edna TH. Short and long-term survival from gastric cancer. A population-based study from a county hospital during 25 years. Acta Oncol. 2007;46:308–15.
74. Schistosomes, liver flukes and *Helicobacter pylori*. IARC Working Group on the evaluation of carcinogenic risks to humans. Lyon, 7–14 June 1994. IARC Monogr Eval Carcinog Risks Hum. 1994;61:1–241.
75. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand. 1965;64:31–49.
76. Nardone G. Review article: molecular basis of gastric carcinogenesis. Aliment Pharmacol Ther. 2003;17 (Suppl 2):75–81.
77. Huang JQ, Sridhar S, Chen Y, Hunt RH. Meta-analysis of the relationship between *Helicobacter pylori* seropositivity and gastric cancer. Gastroenterology. 1998;114:1169–79.
78. Tan VP, Wong BC. Gastric cancer chemoprevention: the current evidence. Gastroenterol Clin N Am. 2013;42:299–316.
79. Lee YC, Chen TH, Chiu HM, Shun CT, Chiang H, Liu TY, et al. The benefit of mass eradication of *Helicobacter pylori* infection: a community-based study of gastric cancer prevention. Gut. 2013;62:676–82.
80. Barker DJ, Coggon D, Osmond C, Wickham C. Poor housing in childhood and high rates of stomach cancer in England and Wales. Br J Cancer. 1990;61:575–8.
81. Roder DM. The epidemiology of gastric cancer. Gastric Cancer. 2002;5 (Suppl 1):5–11.
82. Ladeiras-Lopes R, Pereira AK, Nogueira A, Pinheiro-Torres T, Pinto I, Santos-Pereira R, et al. Smoking and gastric cancer: systematic review and meta-analysis of cohort studies. Cancer Causes Control. 2008;19:689–701.
83. La Torre G, Chiaradia G, Gianfagna F, De Lauretis A, Boccia S, Mannocci A, et al. Smoking status and gastric cancer risk: an updated meta-analysis of case-control studies published in the past ten years. Tumori. 2009;95:13–22.
84. Yoon H, Kim N. Diagnosis and management of high risk groups of gastric cancer. Gut Liver. 2015;9:5–17.
85. de Martel C, Forman D, Plummer M. Gastric cancer: epidemiology and risk factors. Gastroenterol Clin N Am. 2013;42:219–40.
86. Yan S, Li B, Bai ZZ, Wu JQ, Xie DW, Ma YC, et al. Clinical epidemiology of gastric cancer in Hehuang valley of China: a 10-year epidemiological study of gastric cancer. World J Gastroenterol. 2014;20:10,486–94.
87. Dikshit RP, Mathur G, Mhatre S, Yeole BB. Epidemiological review of gastric cancer in India. Indian J Med Paediatr Oncol. 2011;32:3–11.
88. Edgren G, Hjalgrim H, Rostgaard K, Norda R, Wikman A, Melbye M, et al. Risk of gastric cancer and peptic ulcers in relation to ABO blood type: a cohort study. Am J Epidemiol. 2010;172:1280–5.
89. Wang Z, Liu L, Ji J, Zhang J, Yan M, Zhang J, et al. ABO blood group system and gastric cancer: a case-control study and meta-analysis. Int J Mol Sci. 2012;13:13,308–21.
90. Nakao M, Matsuo K, Ito H, Shitara K, Hosono S, Watanabe M, et al. ABO genotype and the risk of gastric cancer, atrophic gastritis, and *Helicobacter pylori* infection. Cancer Epidemiol Biomark Prev. 2011;20:1665–72.
91. Oh S, Kim N, Kwon JW, Shin CM, Choi YJ, Lee DH. Effect of *Helicobacter pylori* eradication and ABO genotype on gastric cancer development. Helicobacter. 2016;21:596–605.
92. Correa P. Gastric cancer: overview. Gastroenterol Clin N Am. 2013;42:211–7.
93. Fallone CA, Barkun AN, Göttke MU, Best LM, Loo VG, Veldhuyzen van Zanten S, et al. Association of *Helicobacter pylori* genotype with gastroesophageal reflux disease and other upper gastrointestinal diseases. Am J Gastroenterol. 2000;95:659–69.
94. Labenz J, Blum AL, Bayerdörffer E, Meining A, Stolte M, Börsch G. Curing *Helicobacter pylori* infection in patients with duodenal ulcer may provoke reflux esophagitis. Gastroenterology. 1997;112:1442–7.
95. Corley DA, Kubo A, Levin TR, Block G, Habel L, Zhao W, et al. *Helicobacter pylori* infection and the risk of Barrett's oesophagus: a community-based study. Gut. 2008;57:727–33.
96. Wang Z, Shaheen NJ, Whiteman DC, Anderson LA, Vaughan TL, Corley DA, et al. *Helicobacter pylori* infection is associated with reduced risk of Barrett's esophagus: an analysis of the Barrett's and esophageal adenocarcinoma consortium. Am J Gastroenterol. 2018;113:1148–55.
97. Vakil N, Hahn B, McSorley D. Recurrent symptoms and gastro-oesophageal reflux disease patients with duodenal ulcer treated for *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2000;14:45–51.
98. Kim N, Lee SW, Kim JI, Baik GH, Kim SJ, Seo GS, et al. Effect of *Helicobacter pylori* eradication on development of reflux esophagitis and gastroesophageal reflux symptoms: a nationwide multicenter prospective study. Gut Liver. 2011;5:437–46.
99. Gasbarrini A, Carloni E, Gasbarrini G, Chisholm SA. *Helicobacter pylori* and extragastric diseases—other Helicobacters. Helicobacter. 2004;9 (Suppl 1):57–66.
100. Lim SH, Kim N, Kwon JW, Kim SE, Baik GH, Lee JY, et al. Positive association between *Helicobacter pylori* infection and metabolic syndrome in a Korean

population: a multicenter nationwide study. Dig Dis Sci. 2019;64:2219–30.

101. Kinjo K, Sato H, Sato H, Shiotani I, Kurotobi T, Ohnishi Y, et al. Prevalence of *Helicobacter pylori* infection and its link to coronary risk factors in Japanese patients with acute myocardial infarction. Circ J. 2002;66:805–10.
102. Fraser A, Scragg R, Cox B, Jackson R. *Helicobacter pylori*, *Chlamydia* pneumoniae and myocardial infarction. Intern Med J. 2003;33:267–72.
103. Danesh J, Wong Y, Ward M, Muir J. Chronic infection with *Helicobacter pylori*, *chlamydia pneumoniae*, or cytomegalovirus: population based study of coronary heart disease. Br Heart J. 1999;81:245–7.
104. Coskun S, Kasirga E, Yilmaz O, Bayindir P, Akil I, Yuksel H, et al. Is *Helicobacter pylori* related to endothelial dysfunction during childhood? Pediatr Int. 2008;50:150–3.
105. Guo Y, Xu C, Zhang L, Chen Z, Xia X. *Helicobacter pylori* infection acts as an independent risk factor for intracranial atherosclerosis in women less than 60 years old. Front Cardiovas Med. 2022;8:819315.
106. Gasbarrini A, Franceschi F. Does *H. pylori* infection play a role in idiopathic thrombocytopenic purpura and in other autoimmune diseases? Am J Gastroenterol. 2005;100:1271–3.
107. Jackson S, Beck PL, Pineo GF, Poon MC. *Helicobacter pylori* eradication: novel therapy for immune thrombocytopenic purpura? A review of the literature. Am J Hematol. 2005;78:142–50.
108. Suzuki T, Matsushima M, Masui A, Watanabe K, Takagi A, Ogawa Y, et al. Effect of *Helicobacter pylori* eradication in patients with chronic idiopathic thrombocytopenic purpura—a randomized controlled trial. Am J Gastroenterol. 2005;100:1265–70.
109. Choe YH, Kim SK, Son BK, Lee DH, Hong YC, Pai SH. Randomized placebo-controlled trial of *Helicobacter pylori* eradication for iron-deficiency anemia in preadolescent children and adolescents. Helicobacter. 1999;4:135–9.
110. Choe YH, Kim SK, Hong YC. *Helicobacter pylori* infection with iron deficiency anaemia and subnormal growth at puberty. Arch Dis Child. 2000;82:136–40.
111. Kim SB, Kim N, Park J, Hwang IC, Lim SH, Song DH, et al. Preventive effect of *Helicobacter pylori* eradication on the coronary heart diseases depending on age and sex with a median follow-up of 51 months. Helicobacter. 2023;28:e12969.
112. Chey WD, Wong BC. Practice parameters Committee of the American College of gastroenterology. American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection. Am J Gastroenterol. 2007;102:1808–25.

18

Atrophic Gastritis and Intestinal Metaplasia

Nayoung Kim and Yo Han Park

Abstract

Helicobacter pylori (*H. pylori*) infection causes atrophic gastritis (AG) and intestinal metaplasia (IM), which are known as the main precursor lesions of gastric cancer (GC). The prevalence of AG and IM varies depending on countries, even it represents diverse results in the same nation. Usually AG is antecedent of IM, but the risk factors of AG and IM are not always the same. Furthermore, the management strategy of AG and IM has not been established, yet. However, eradication of *H. pylori* is very important to prevent the progression of AG and IM to GC. Therefore, early diagnosis of AG and IM is very important, especially in high incidence area of GC. However, as the endoscopic, histological, and serological diagnostic tool had a limitation, a multifactorial assessment is needed to ameliorate the diagnostic accuracy of AG and IM.

Keywords

Atrophic gastritis · Intestinal metaplasia · *Helicobacter pylori* · Premalignant lesion

18.1 Introduction

Helicobacter pylori (*H. pylori*) causes progressive gastric damage that is initially most prominent in the antrum and advances into the corpus [1, 2]. Gastric cancer (GC) risk is associated with the extent and severity of atrophic injury, which is recognized by loss of normal glandular elements (atrophy), and the development of metaplastic epithelia (pseudopyloric or spasmolytic polypeptide-expressing type and intestinal type) [1, 3, 4]. The risk of developing GC increases with the rate of development of these atrophic changers [1]. In Western countries such as the United States, the incidence of GC was noted to decline rapidly such that it fell from being the most common cancer in the first quarter of the twentieth century to an uncommon disease by the beginning of the twentieth century [5]. The different *H. pylori*-related diseases are associated with different patterns of gastritis (i.e., atrophic pangastritis or corpus-predominant gastritis in gastric ulcer and GC, and antral-predominant with duodenal ulcer) suggesting that the rapid changes in disease manifestation were accompanied by similar changes in the rate of development of atrophic gastritis (AG) [1, 3–7]. When

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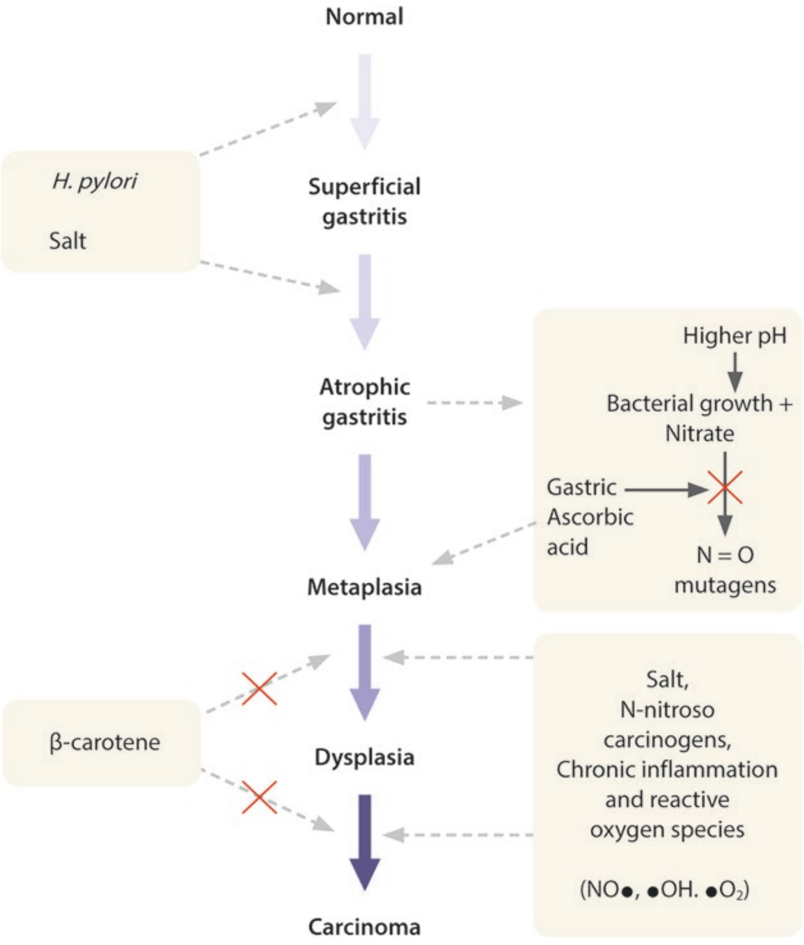
the normal gastric mucosa is replaced by mucosa which resembles that of the intestine, this condition is called as intestinal metaplasia (IM). As the GC risk increases very rapidly, AG and IM are considered to be premalignant lesions of GC. For this reason, effective diagnosis and management of AG and IM is a very important research topic to prevent GC [8, 9]. According to a recent meta-analysis, the incidence rates of AG are in a wide range from 0% to 10.9% per year [10]. This wide range of incidence of AG could be explained by the different settings in which the diagnoses of AG were made [10]. *H. pylori* infection is the most important risk factor of AG and IM. As AG is considered to be an antecedent to IM [11]; thus, the risk factors for AG are expected to be similar to those for IM. However, bacterial factors of *H. pylori* have been found to play an important role for AG, while environmental and host factors are more important for IM [11]. From this background, the aim of this chapter is to provide comprehensive information regarding the epidemiology, etiology, diagnosis, and management of AG and IM, especially in regard to *H. pylori*, which will lay a foundation to establish strategies to prevent GC.

18.2 Atrophic Gastritis and Intestinal Metaplasia as Precursor Lesions of Gastric Cancer

Chronic inflammation can damage inflamed cells and trigger a multistep process of carcinogenesis. In premalignant tissues associated with chronic inflammation, tumor cells and leukocytes of various kinds such as neutrophils, macrophages, monocytes, mast cells, eosinophils, dendritic cells, and lymphocytes are present [12, 13]. These inflammatory cells contribute to cancer initiation, promotion, and metastasis by producing cytokines, reactive oxygen species, and reactive nitrogen species. Various oxidant products can damage cellular DNA, RNA, and proteins by chemical reactions such as oxidation, nitration, nitrosation, and halogenation. Damages of cellular components result in increased mutations and altered functions of important enzymes and proteins in premalignant tissues, so contributing to the multistage carcinogenesis process [14]. According to the Correa model, chronic inflammation of gastric mucosa triggers a pathway of chronic active gastritis, multifocal atrophy, IM, gastric dysplasia, and finally invasive gastric adenocarcinoma [15] (Fig. 18.1). The pathogenesis of intestinal-type GC can be explained by a multistep process from chronic gastritis through AG, IM, and dysplasia to cancer. The presence of AG, which has been traditionally defined as the loss of glands [16], is well known as a risk factor of GC. The risk of GC increases with greater extent and higher degree of gastric mucosal atrophy [17]. Gastric IM is defined as the replacement of the surface, foveolar, and glandular epithelium in the gastric mucosa by intestinal epithelium with the presence of Paneth cells, goblet cells, and absorptive cells [17]. Several studies suggest that AG and IM are major precursor lesions of GC [3, 18, 19]. These relationships were observed before the identification of *H. pylori* infection in 1982 [20]. GC is divided into two major histological types, intestinal type and diffuse type, by Lauren classification [21]. Intestinal-type cancers are believed to arise secondary to AG and IM [22]. On the contrary, diffuse-type GC usually arises independently of IM. This raised doubts about the association between IM and GC development [23]. It is thought that diffuse type is more likely to have a primary genetic etiology, and the involvement of *H. pylori* is probably limited to a subset of sporadic cases [22, 23]. However, many studies suggest that both intestinal and diffuse types of gastric carcinoma are associated with *H. pylori* infection [24, 25]. Our team also reported that high-risk atrophy and IM were found to be important prediction markers for GC not only for the intestinal-type GC but also for diffuse-type GC [26].

Compared with other risk factors of GC, AG and IM increase the risk of intestinal-type GC exponentially. That is, the risk of GC in subjects with severe fundal AG was 5.76 times higher than that in those having little or no fundal AG [27]. In case of IM, it increased risk of developing GC more than tenfold [28]. In a prospective study of

Fig. 18.1 Correa's theory regarding human gastric carcinogenesis: a multistep and multifactorial process. Gastritis begins from superficial gastritis and progresses into atrophic gastritis, metaplasia, dysplasia, and intestinal-type gastric cancer (Modified from Correa [15])


Normal
H. pylori
Salt
Superficial gastritis
Atrophic gastritis
Higher pH
Bacterial growth + Nitrate
Gastric Ascorbic acid
N = O mutagens
Metaplasia
β-carotene
Dysplasia
Salt, N-nitroso carcinogens, Chronic inflammation and reactive oxygen species
(NO●, ●OH. ●O2)
Carcinoma

1526 subjects, 1246 patients had *H. pylori* infection and 280 did not [3]. During mean follow-up of 7.8 years, GC developed in the individuals (36 patients, 2.9%) infected with *H. pylori* but not in the uninfected persons [3]. Histological severe gastric atrophy, corpus-predominant gastritis, or IM were increasing risk factors of GC [3]. In addition, in a cohort of 4655 healthy asymptomatic subjects, the risk of GC increased stepwise from chronic atrophic gastritis (CAG)-free gastritis [*H. pylori*(+)/CAG(−) group] (hazard ratio [HR] 7.13; 95% confidential interval [CI], 0.95–53.33) to CAG [*H. pylori*(−)/CAG(+) group] (HR 14.85; 95% CI, 1.96–107.7) and finally to severe CAG with extensive IM [*H. pylori*(−)/CAG(+)group] (HR 61.85; 95% CI, 5.6–682.64) in which *H. pylori* was lost [18]. Probably *H. pylori* alone is not directly associated with gastric carcinogenesis. Recently the interplay between *H. pylori* and gastrointestinal microbiota is well known [29]. As *H. pylori* produces very powerful toxins such as CagA or VacA, *H. pylori*-induced chronic inflammation can provide the seed of cascade leading to GC, which can progress continuously even in the absence of *H. pylori* [30]. The patients with *H. pylori* infection and IM had more than 6.4-fold increased risk of GC than the subjects of infected with *H. pylori* infection but without IM [3]. In a cohort study of 2224 subjects conducted in South Korea, the group with IM had 10.9-fold increased risk of GC [19]. *H. pylori* infection triggers a multistep progression from chronic gastritis, gastric atrophy, IM, and finally into GC [31]. *H. pylori*, a gram-negative, flagellated bacterium about 3 μm long with a diameter of about 0.5 μm [32], became the first bacterium to be classified as a type I carcinogen by IARC working group in 1994 [33]. In a meta-analysis of 19 cohort or case-control studies, the summary odds ratio (OR) for GC in *H. pylori*-infected subjects was 1.92 (95% CI, 1.32–2.78) [34]. In another meta-analysis of 42 cohort or case-control studies, the summary OR for *H. pylori* infection in relation to gastric carcinoma was 2.04 (95% CI, 1.69–2.45) [35]. So, these studies show the clear association between *H. pylori* and gastric adenocarcinomas [34, 35]. However, among *H. pylori*-positive patients, only 1–2% subjects [17] or 3% subjects develop GC [36], suggesting that the final effects of *H. pylori* infection could be determined by prevalence, environmental factors, bacterial factors, and host factors [37]. Despite the clear relationship between *H. pylori* infection and gastric adenocarcinomas through AG and IM, especially in the intestinal type of GC, the mechanisms of process about chronic inflammation and developing GC are under investigation. The enhanced production of free radicals by chronic *H. pylori* infection causes mutations in target cells so the neoplastic clones are established [38]. In addition, tumor necrosis factor (TNF)-α plays major roles in the growth, invasion, and metastasis of neoplasm, and this mechanism is called as a "perigenetic pathway" [38]. Furthermore, TNF-α-inducing protein (Tipα) from *H. pylori* binds to and enters the nucleus through a specific binding molecule, which might act as a carcinogen of GC [39].

18.3 Prevalence of Atrophic Gastritis and Intestinal Metaplasia

A meta-analysis of 14 studies on incidence of AG was reported in 2010 [10], which showed the incidence rates of AG ranged from 0% to 10.9% per year [10]. This wide range of incidence could be explained by the particular settings in which the diagnosis of AG was made [10]. The lowest incidence rates (0%) were found in patients with reflux esophagitis and in patients successfully treated for *H. pylori* infection [40, 41]. The highest incidence rate (10.9%) was observed in a study conducted in patients who underwent vagotomy for ulcer disease [42]. Regarding the *H. pylori* infection, the AG incidence rate was higher in the *H. pylori*-positive patients than in the *H. pylori*-negative ones [10]. In a meta-analysis, rate ratios comparing the incidence of AG in *H. pylori*-positive patients to that in *H. pylori*-negative ones ranged from 2.4 to 7.6 with a summary estimate of 5.0 (95% CI, 3.1–8.3) [10]. In contrast, reports on the incidence of IM are rare in asymptomatic general population, because upper gastrointestinal endoscopy with histological examination is needed to estimate the prevalence of IM. In a study about the relationship between IM and *H. pylori* infection in the Netherlands, IM was found more often in *H. pylori*-positive patients than in *H. pylori*-negative ones (33.9% vs. 15.2%, $p < 0.001$) [43]. The mean age of IM-positive patients was 64 years and that of IM-negative ones was 72 years ($p < 0.005$) [43]. In a Japanese prospective study with a follow-up of 7.8 years, IM was detected in as many as 37% of 1426 *H. pylori*-positive patients (mean age: 52.3 years) while in only 2% of 280 uninfected patients (mean age: 52.7 years) [3]. There were several studies regarding the prevalence of AG and IM in South Korea, where the incidence of GC is very high. In a cohort study consisted of 389 subjects (≥16 years), the prevalence of AG in the antrum and corpus was 42.5% and 20.1%, and the prevalence of IM was 28.6% and 21.2%, respectively [11]. In another study reporting age-adjusted prevalence in South Korea, the prevalence of AG was 42.7% for males and 38.1% for females ($p = 0.194$) and that of IM was 42.5% for males and 32.7% for females ($p = 0.005$) [44]. The prevalence of AG and IM increased significantly with age for both males and females [44]. When a multicenter study of South Korea in 2006, with 25,536 asymptomatic subjects, evaluated the prevalence of endoscopic AG and IM, they were 27.1% and 7.1%, respectively, which were lower than those estimated with histological diagnoses [45]. The proportions of endoscopic AG and IM in the patients aged 40 years or less were 14.9% and 2.7%, respectively, and in those aged 60 years or more were 43.5% and 12.3%, respectively [45]. This study showed that the prevalence of AG and IM increased significantly with age and more frequent in males than in females [45]. Interestingly, the prevalence of endoscopic AG and IM were 40.7% and 12.5% in a multicenter study of 4023 subjects in 2011, significantly higher than those of a multicenter study in 2006 [46]. This increase in prevalence of AG and IM may be attributed to aggressive diagnosis made by physicians in 2011 relative to 2006 instead of real increase of prevalence rate of AG and IM [46]. To investigate the trends of atrophy and IM, our team prospectively enrolled 2002 subjects without significant gastroduodenal diseases and divided into three periods (2003-2007, 2008–2012, and 2013–2018) [47]. *H. pylori*-negative and *H. pylori*-positive subjects were 1220 (61.0%) and 782 (38.0%), respectively. *H. pylori* positivity decreased from 149/303 (49.2%), 207/515 (40.2%), and 426/1184 (36.0%), in the three periods, respectively ($p < 0.001$) [47]. The prevalence of atrophy ($p < 0.001$) and IM in the corpus ($p < 0.001$) significantly decreased over 15 years in females, but not in males [47] (Fig. 18.2, Table 18.1). The mean grade of atrophy and IM was higher in males (0.36 and 0.51) than in females (0.28 and 0.41) in the corpus ($p = 0.027$) and in the antrum ($p = 0.006$), respectively (Fig. 18.2, Table 18.1). Similarly, the mean grade of IM in males (0.34) was higher in females (0.19; $p < 0.001$) in the corpus [47] (Fig. 18.2 and Table 18.1). These results suggest a significant decrease in atrophy and IM in the corpus in females over 15 years depending on sex- or gender-specific manners [47].

In case of Japan, the prevalence of histological atrophy in the antrum and in the corpus was significantly lower in the 2010s (33% and 19%, respectively) compared to those evaluated in either the 1970s (98% and 82%, respectively) ($p < 0.001$) or 1990s (80% and 67%, respectively) ($p < 0.001$) [48]. The severity of atrophy and IM also declined remarkably among those with *H. pylori* infection [48]. Actually, the prevalence of AG and IM varied (9.4–63.8% in case of AG; 7.1–42.5% in case of IM) depending on diagnostic methods and countries [4, 11, 41–50] (Table 18.1).

18.4 Risk Factors of Atrophic Gastritis and Intestinal Metaplasia

Several studies have suggested that dietary causes such as excessive salt intake, deficient ascorbic acid, and insufficient carotene could be risk factors of AG, IM, and GC [15, 55]. However, among the many risk factors of AG and IM, *H. pylori* infection was found as the most important [3, 18, 19, 33]. Especially, bacterial virulence genes such as *cagA* and *vacA* were found to be the important risk factors of AG, IM, and GC [56–60]. In a study of 58 subjects of *H. pylori* infection, with a mean follow-up period of 11.5 years, *cagA* was associated with a significant risk of AG and IM (OR 3.48; 95% CI, 1.02–12.18) [57]. Infection with *cagA*-positive strains further increased the risk for GC by 1.64 (95% CI, 1.21–2.24) overall in a meta-analysis [58]. CagA protein secreted by *H. pylori* translocates into cytoplasm of gastric mucosa cells via its type IV secretion system after *H. pylori* attachment. CagA interrupts signaling pathways by phosphorylation-dependent and phosphorylation-independent mechanisms, leading to cytoskeletal change, motility, and abnormal proliferation in normal gastric epithelial cells [37]. *vacA* gene is another well-known virulence factor, s1 and m1 forms of *vacA* are more common in disease-associated *H. pylori* strains, and s1m1 genotype was related to gastric epithelial damage, AG, and IM [56, 59, 60]. From a study of a total of 370 *H. pylori*-infected patients, a high association was observed between the presence of AG or IM and the prevalence of *vacA* s1, m1, and *cagA* strains of *H. pylori* genotypes [60]. That is, the presence of atrophy was associated with *vacA* s1 (OR 46.9; 95% CI, 8.6–256.4), *vacA* m1 (OR 10.1; 95% CI, 4.3–23.7), and *cagA* (OR 11.2; 95% CI, 4.5–27.8) [60]. Similarly, presence of IM was associated with *vacA* s1 (OR 14.9; 95% CI, 4.2–53.2), *vacA* m1 (OR 6.8; 95% CI, 2.9–15.9), and *cagA* (OR 4.8; 95% CI, 2.1–10.6) [60]. Furthermore, other virulence factors such as SabA (outer membrane protein) were significantly associated with AG, IM, and GC [61]. Many studies showed that the *vacA* and *cagA* genotypes of *H. pylori* are not

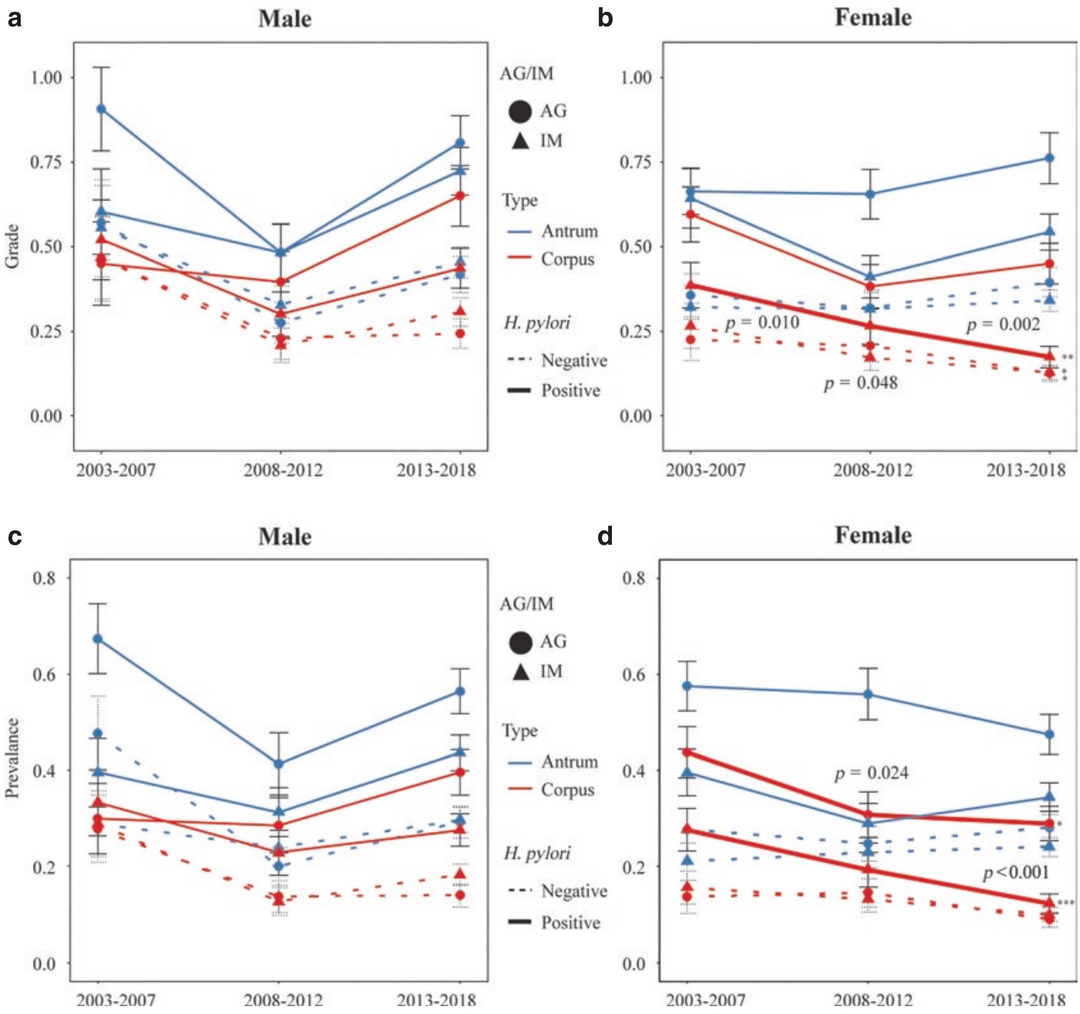


Fig. 18.2 Trends of grades and prevalence of atrophy and intestinal metaplasia (IM) according to biopsy site (antrum and corpus) and *H. pylori* infection status over 15 years. (**a**) In males, grades of atrophy and IM did not show any significant differences during the three periods. (**b**) In females, grades of atrophy and IM in the corpus of *H. pylori*-negative subjects were statistically significant ($p = 0.048$ and $p = 0.010$). In addition, grades of IM in the corpus of *H. pylori*-positive subjects were statistically significant ($p = 0.002$). (**c**) In males, the prevalence of atrophy and IM was significantly different during the three periods. (**d**) In females, the prevalence of atrophy and IM in the corpus of *H. pylori*-positive subjects was statistically significant ($p = 0.024$ and $p < 0.001$). A linear-by-linear association test was used for the analysis (Adapted from Kwon et al. [47])

equally distributed all over the world [62–64]. There are significant differences in *H. pylori* genotypes among populations from Asia, different parts of Europe, and North and South America. Given the geographic distribution of specific *H. pylori* genotypes, it could be speculated that there might be differences in incidence of GC [62–64].

In a single-center prospective study in 389 subjects without significant gastroduodenal diseases in South Korea, risk factors of histological AG in the antrum and in the corpus were *H. pylori* infection, *cagA*, and *vacA* m1 positivity and age ≥61 years [11] (Table 18.2). The risk factors of histological IM in the antrum and in the corpus were age ≥61 years, *H. pylori* infection,

Table 18.1 The prevalence of atrophic gastritis and intestinal metaplasia in the world

Author, year	Country	Diagnostic methods	Study population (*n*)	AG (%) antrum/corpus	IM (%) antrum/corpus
Kim et al. (2008) [11]	South Korea	Histology	389	42.5/20.1	28.6/21.2
Kim et al. (2008) [44]	South Korea	Histology	713	42.7/38.1	42.5/32.7
Park et al. (2012) [45]	South Korea	Endoscopy	25,536	27.1[a]	7.1[a]
Joo et al. (2013) [46]	South Korea	Endoscopy	4023	40.7[a]	12.5[a]
Kwon et al. (2021) [47]	South Korea	Histology	303 (2003–2007) Male: 93 Female: 210	Total: 46.8/28.1	Total: 31.4/28.1
				Male: 57.6/28.9	Male: 34.4/31.2
				Female: 42.0/27.7	Female: 30.0/21.4
			515 (2008–2012) Male: 217 Female: 298	Total: 32.7/20.2	Total: 26.0/16.1
				Male: 27.6/19.2	Male: 26.7/16.6
				Female: 36.4/21.0	Female: 25.5/15.8
			1184 (2013–2018) Male: 480 Female: 704	Total: 36.9/18.3	Total: 30.7/15.2
				Male: 39.5/22.3	Male: 34.8/21.7
				Female: 35.0/15.6	Female: 27.8/10.8
Weck et al. (2007) [48]	Germany	Serology[b]	9444	6.0[a]	
Borch et al. (2000) [49]	Sweden	Histology	501	9.4[a]	
Asaka et al. (2008) [50]	Japan	Histology	2455	55.5[a]	24.2[a]
Kamada et al. (2014) [4]	Japan	Histology	216 (1970s)	98/82[a]	63.9/32.4[a]
			417 (1990s)	80/67[a]	37.4/21.3[a]
			107 (2010s)	33/19[a]	15.0/4.7[a]
Zou et al. (2011) [51]	China	Histology	1022	63.8[a]	
Eriksson et al. (2008) [52]	Finland	Histology	505		18.8/7.1
Almouradi et al. (2013) [53]	USA	Histology	437		15.0[a]

Modified from Park and Kim [54]

AG Atrophic gastritis, *IM* Intestinal metaplasia, *USA* United States of America

[a] Prevalence of atrophic gastritis or intestinal metaplasia in the antrum and/or corpus

[b] Serology by *H. pylori* IgG antibodies

strong spicy food, a smoking history, and the presence of *IL10-592* C/A as against A/A [11] (Table 18.3). Finally, virulence factor of *H. pylori* was the most important risk factor of AG, while environmental or host factors were more important risk factors for IM, which suggests a kind of difference of risk factors between AG and IM [11]. In another study on the risk factors of endoscopic AG and IM in the 4023 health checkup subjects, the risk factors for AG were male, age groups of 40–59 years and >60 years, positive *H. pylori* serology, IM, and education below the college level (OR; 5.00, 2.55, 1.38, 1.41, 4.29, and 1.35, respectively) [46] (Table 18.4). The risk factors for IM were male, AG, age groups of 40–59 years and >60 years, positive *H. pylori* serology, having relatives with GC, consumption of dairy products, and education below the college level (OR; 3.25, 2.17, 3.16, 1.88, 3.68, 1.48, 1.40, and 1.47, respectively) [46] (Table 18.5).

Table 18.2 Risk factors of histological atrophic gastritis by multivariate analysis

Antrum	AG grade 0[a]	AG grade 1–3[a]	Adjusted OR	95% CI	*p* value
H. pylori (%)					
Negative	56 (83.6)	11 (16.4)			
Positive	55 (43.7)	71 (56.3)	5.69	1.87–17.3	<0.001
Age (years) (%)					
47	58 (69.9)	25 (30.1)			
48–60	32 (48.5)	34 (51.5)	2.23	0.87–5.70	0.094
≥61	21 (47.7)	23 (52.3)	3.75	1.31–10.9	0.014
cagA (%)					
Negative	52 (64.2)	29 (35.8)			
Positive	22 (40.7)	32 (59.3)	2.67	1.05–6.84	0.040
Corpus	AG grade 0[a]	AG grade 1–3[a]	Adjusted OR	95% CI	*p* value
H. pylori (%)					
Negative	84 (91.3)	8 (8.7)			
Positive	115 (73.2)	42 (26.8)	3.63	1.58–8.34	0.003
Age (years) (%)					
≤47	91 (90.1)	19 (9.9)			
48–60	64 (74.4)	22 (25.6)	3.90	1.36–11.2	0.011
≥61	44 (71.0)	18 (29.0)	4.94	1.61–15.2	0.005
vagA m (%)					
Negative	80 (84.2)	15 (15.8)			
m1	37 (62.7)	22 (37.3)	3.48	1.54–7.87	0.003
m2	9 (83.3)	1 (16.7)	1.04	0.10–11.0	0.973

Adapted from Kim et al. [11]

AG Atrophic gastritis, *OR* Odds ratio, *CI* Confidence interval, *H. pylori Helicobacter pylori*

[a] Updated Sydney system scores, 0 = none, 1 = slight, 2 = moderate, 3 = marked

Table 18.3 Risk factors of histological intestinal metaplasia by multivariate analysis

Antrum	IM grade 0[a]	IM grade 1–3[a]	Adjusted OR	95% CI	*p* value
H. pylori (%)					
Negative	119 (89.5)	14 (10.5)			
Positive	156 (61.9)	96 (38.1)	8.22	3.27–20.7	<0.001
Age (years) (%)					
≤47	133 (88.1)	18 (11.9)			
48–60	102 (78.5)	28 (21.5)	2.28	1.04–4.98	0.039
≥61	69 (65.7)	36 (34.3)	3.02	1.34–6.78	0.008
Smoking (%)					
Never	145 (81.0)	34 (19.0)			
Current	33 (91.7)	3 (8.3)	0.39	0.05–4.58	0.372
Past	46 (67.6)	22 (32.4)	3.49	1.3–8.75	0.008
Spicy food (%)					
Low	51 (79.7)	13 (20.3)			
Moderate	90 (70.3)	38 (29.7)	0.78	0.66–1.34	0.788
Strong	37 (58.7)	26 (41.3)	2.38	1.16–4.88	0.017
IL6-572 genotype (%)					
C/C	92 (64.8)	50 (14.3)			
G carrier	88 (74.6)	30 (25.4)	0.50	0.26–0.94	0.033
Corpus	IM grade 0[a]	IM grade 1–3[a]	Adjusted OR	95% CI	*p* value
H. pylori (%)					
Negative	122 (91.7)	11 (8.3)			
Positive	182 (71.9)	71 (28.1)	3.65	1.51–8.87	0.004

Table 18.3 (continued)

Antrum	IM grade 0[a]	IM grade 1–3[a]	Adjusted OR	95% CI	*p* value
Age (years) (%)					
≤47	133 (88.1)	18 (11.9)			
48–60	102 (78.5)	28 (21.5)	2.47	0.96–6.35	0.061
≥61	69 (65.7)	36 (34.3)	9.98	3.78–26.3	0.005
Smoking (%)					
Never	235 (78.8)	51 (21.2)			
Current	39 (92.9)	3 (7.1)	0.18	0.02–1.34	0.093
Past	21 (46.7)	24 (53.3)	7.10	2.48–20.3	<0.001
Occupation (%)					
Professional	103 (81.1)	24 (18.9)			
Nonprofessional	43 (72.9)	16 (27.1)	3.53	1.17–10.7	0.025
Unemployed	20 (64.5)	11 (35.5)	3.79	1.28–11.2	0.017
IL10-592 genotype (%)					
A/A	91 (82.0)	20 (18.0)			
C/A	83 (68.6)	38 (31.4)	3.69	1.65–8.21	0.001
C/C	24 (75.0)	8 (25.0)	1.32	0.41–4.21	0.639

Adapted from Kim et al. [11]
IM Intestinal metaplasia, *OR* Odds ratio, *CI* Confidence interval, *H. pylori Helicobacter pylori*
[a] Updated Sydney system scores, 0 = none, 1 = slight, 2 = moderate, 3 = marked

Table 18.4 Risk factors of endoscopic atrophic gastritis by multivariate analysis

Variables	Estimate	SE	*p* value	OR	95% CI
Age (years)					
<40				1.00	
40–59	1.039	0.165	<0.001	2.55	2.05–3.18
≥60	1.980	0.203	<0.001	5.00	3.71–6.74
Male	0.603	0.155	<0.001	1.38	1.17–1.64
H. pylori IgG positivity	0.377	0.112	<0.001	1.41	1.19–1.66
Intestinal metaplasia	1.309	0.147	<0.001	4.29	3.35–5.50
Education below college	−0.131	0.216	0.046	1.35	1.01–1.79

Adapted from Joo et al. [46]
SE Standard error, *OR* Odds ratio, *H. pylori Helicobacter pylori*, *CI* Confidence interval

Table 18.5 Risk factors of endoscopic intestinal metaplasia by multivariate analysis

Variables	Estimate	SE	*p* value	OR	95% CI
Age (years)					
<40				1.00	
40–59	1.150	0.205	<0.001	3.16	2.11–472
≥60	1.178	0.236	<0.001	3.25	2.05–5.15
Male	0.631	0.154	<0.001	1.88	1.39–2.54
H. pylori IgG positivity	0.775	0.119	<0.001	2.17	1.72–2.74
Atrophic gastritis	1.303	0.113	<0.001	3.68	2.95–4.60
Relatives of gastric cancer	0.395	0.143	0.006	1.48	1.12–1.96
Smoking	0.170	0.138	NS	1.19	0.91–1.55
Alcohol	0.112	0.131	NS	1.20	0.87–1.45
Education below college	-0.384	0.162	0.018	1.47	1.06–2.00
Consumption of dairy product	0.338	0.116	0.004	1.40	1.12–1.76

Adapted from Joo et al. [46]
SE Standard error, *OR* Odds ratio, *H. pylori Helicobacter pylori*, *NS* Not significant, *CI* Confidence interval

When our team multivariate analysis regarding the risk factors of AG and IM in the 2002 subjects without significant gastroduodenal diseases it showed that old age, and *H. pylori* were statistically significant in atrophy of antrum and corpus, and IM in the corpus [47]. In cases of IM of antrum, old age, *H. pylori*, and smoking were statistically significant [47].

18.5 Classification of Atrophic Gastritis and Intestinal Metaplasia

Although several classifications of gastritis have been proposed, there is no universally accepted classification of gastritis. The first classification based on histopathological examination of gastric mucosa collected by so-called blind biopsies and samples collected during surgical procedures was created by Schindler [65]. Schindler described that gastritis was divided into a superficial gastritis (Fig. 18.3b) that may progress to AG (Fig. 18.3c) and IM (Fig. 18.3d) with time, which is different from normal (Fig. 18.3a). Additionally, Schindler proposed that there were different courses and prognoses of disease by the type of the gastritis [65]. And a novel classification and grading of gastritis were devised by a group of experts at the 9th World Congress of Gastroenterology in Sydney, Australia, in 1990 [66]. The histological division of Sydney system is a practical guideline upon which the morphological features of gastritis in endoscopic biopsy samples should be documented. Type, severity, and extent of gastric inflammation composed to possible etiology should be detailed depending on the chart. The Sydney system asserted the routine gastric biopsy sampling protocol (two from the antrum and two from the corpus, both from anterior and posterior walls) and sample fixation in adequately labeled separate containers [66]. Now the updated Sydney system in 1996 is most widely used as the classification of gastritis [67]. The original Sydney classification of gastritis dividing into gastritis acute, chronic, and special forms and grading of chronic inflammation, neutrophil activity, atrophy, IM, and *H. pylori* density into mild, moderate, and marked categories were kept [66]. The updated Sydney system introduced a visual analog scale for evaluating the severity of histological grading [67] (Fig. 18.4). The histological features of the gastric mucosa were recorded using updated Sydney system scores, that is, 0 = none, 1 = mild, 2 = moderate, and 3 = marked [67]. It changed the routine of endoscopic biopsy sampling by the introduction of biopsy sampling from the incisura angularis and modified the corpus and antrum biopsy locations from the two opposite

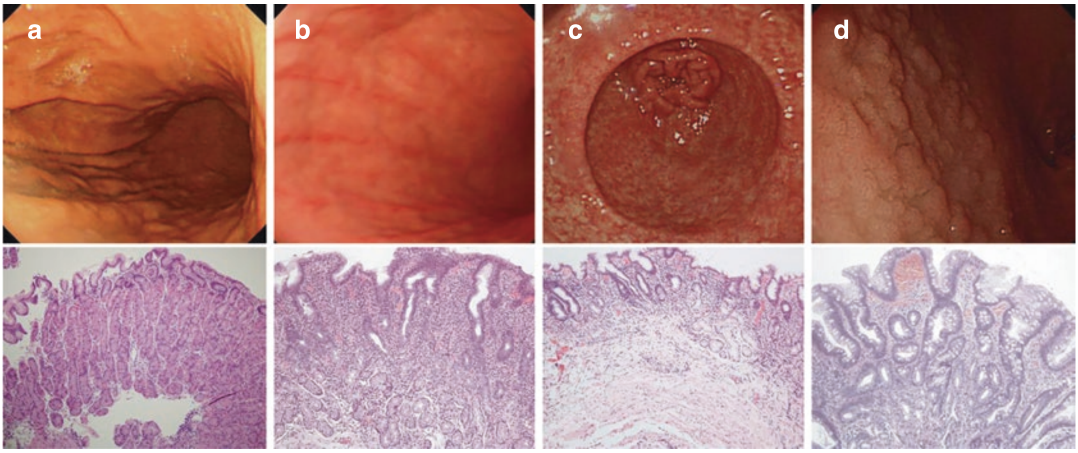


Fig. 18.3 Classification of gastritis (**a**) normal, (**b**) superficial gastritis, (**c**) atrophic gastritis, and (**d**) intestinal metaplasia. Upper panel is endoscopic and lower panel is histologic finding (Adapted from Park and Kim [54])

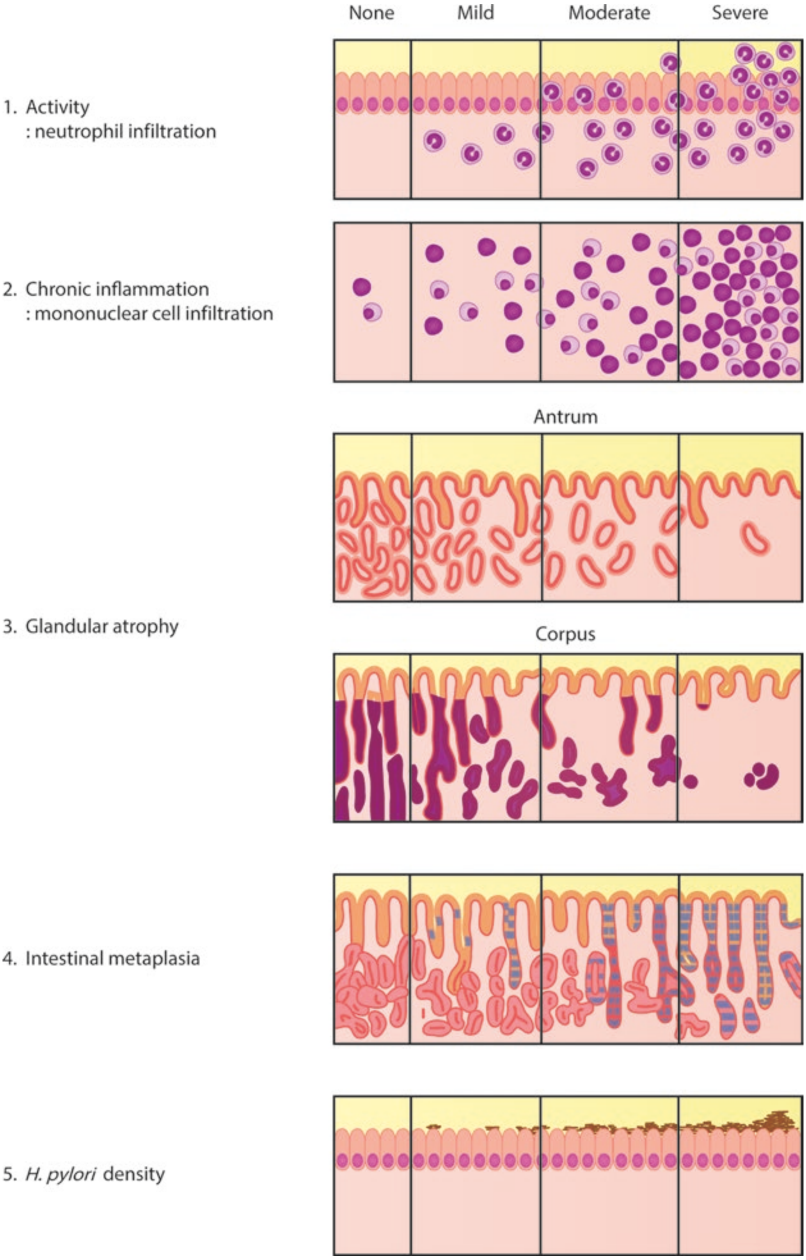

None
Mild
Moderate
Severe
1. Activity
: neutrophil infiltration
2. Chronic inflammation
: mononuclear cell infiltration
Antrum
3. Glandular atrophy
Corpus
4. Intestinal metaplasia
5. *H. pylori* density

Fig. 18.4 Grading of gastritis by Sydney system: acute inflammation, chronic inflammation, atrophic gastritis, intestinal metaplasia, and *H. pylori* density (Adapted from Dixon et al. [67])

walls in lesser and greater curvature of both parts [67] (Fig. 18.5). Endoscopic description of acute gastritis by Sydney system is divided into edema, exudates, erosions, and hemorrhage. In contrast, endoscopic diagnosis and classification of chronic gastritis are reliable depending on the interobserver [67]. In addition, recently Kyoto global consensus for gastritis was developed based on etiology-based classification of gastritis and duodenitis, for international classification system as well as further research [68].

However, all of these classifications do not reflect prognosis of GC risk in subjects with CAG. Therefore, the Operative Link on Gastritis Assessment (OLGA) was developed to improve the histological staging system for gastric atrophy [69]. OLGA system uses gastric biopsy sampling protocol defined by Sydney system and the

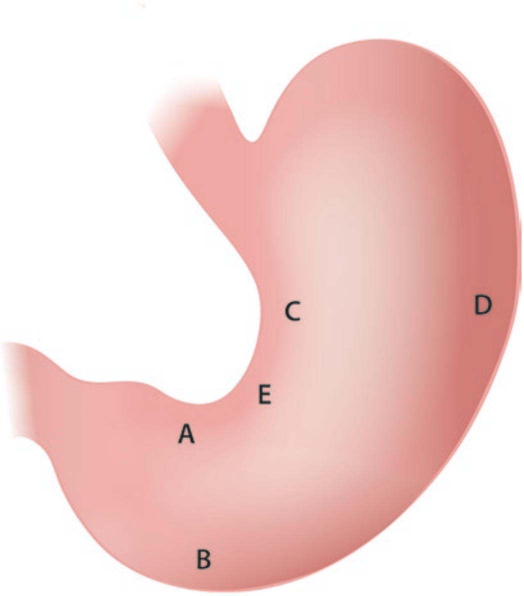
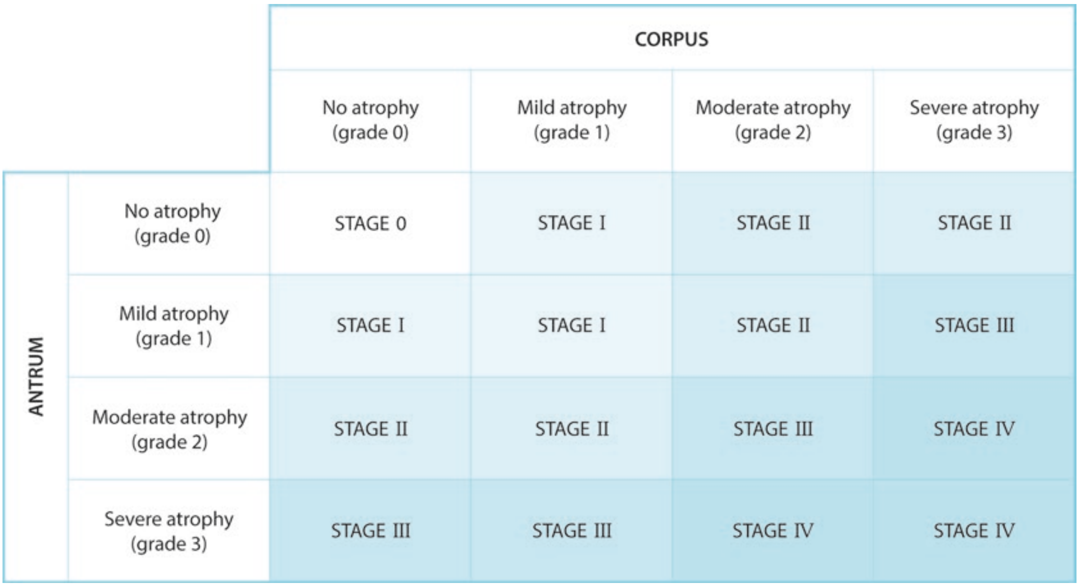


Fig. 18.5 The optimal gastric biopsy sites recommend by updated Sydney system. Biopsy specimens are taken at five different sites: (**A**) lesser curvature of the antrum; (**B**) greater curvature of the antrum; (**C**) lesser curvature of the corpus; (**D**) greater curvature of the corpus; and (**E**) incisura angularis (Adapted from Dixon et al. [67] and Park and Kim [54])

histological grading system recommended by the updated Sydney system [69] (Fig. 18.6). Long-term follow-up studies with follow-up ranges from 144 to 204 months proved that the OLGA staging reflects relevant information on clinicopathological outcome of gastritis, and therefore *H. pylori*-negative patients with low OLGA stages could be excluded from secondary preventive surveillance of GC [70], whereas patients with higher OLGA stages (stages III and IV) should be considered definitely candidates for endoscopic examination [70]. However, there are no universally acceptable classification methods for gastritis, yet. For the optimization of the methods to interpret gastritis, repetitive communication between the endoscopists and pathologists should be required.

IM could be classified according to the phenotype of mucin, and it will be touched more in detail in the histological diagnosis part of this chapter. Type I IM expresses only sialomucins, type III expresses sulfomucins, and type II expresses a mixture of gastric and intestinal mucins [71]. Several studies reported that type III



		CORPUS			
		No atrophy (grade 0)	Mild atrophy (grade 1)	Moderate atrophy (grade 2)	Severe atrophy (grade 3)
ANTRUM	No atrophy (grade 0)	STAGE 0	STAGE I	STAGE II	STAGE II
	Mild atrophy (grade 1)	STAGE I	STAGE I	STAGE II	STAGE III
	Moderate atrophy (grade 2)	STAGE II	STAGE II	STAGE III	STAGE IV
	Severe atrophy (grade 3)	STAGE III	STAGE III	STAGE IV	STAGE IV

Fig. 18.6 Gastritis staging: the operative link on gastritis assessment (OLGA) system. Atrophy is defined as loss of appropriate glands (with or without metaplasia). In each compartment, atrophy is scored in a four-tiered scale (0–3) according to the visual analog scale of the updated Sydney system (Adapted from Rugge et al. [69])

or incomplete IM increases the risk of GC [72, 73]. However, a contrary result has been published [74]. Furthermore, IM subtyping was not found to play a major role in the prediction of GC development in South Korea [75]. International guideline did not recommend subtyping of IM for clinical practice in 2012 [76]. However, a recent systemic review concluded that the incomplete IM was significantly related to the prevalence of GC [77]. Moreover, it reported that the relative risks of GC were from 4- to 11-fold higher for the presence of incomplete type in comparison to complete type or in comparison to the absence of incomplete type [77]. This systemic report concluded that subtyping of IM has the scientific evidence on the evaluation of GC risk [77].

18.6 Diagnosis of Atrophic Gastritis and Intestinal Metaplasia

In most of previous studies about the clinical relevance of endoscopic and histological diagnosis of the gastritis, the diagnosis of gastritis should have been based on histological examination of the gastric mucosa [78, 79]. Especially in the young age group, a high index of suspicion of gastric atrophy is important, and confirmation of the diagnosis by histology is necessary [80]. However, in another study, endoscopic and histological diagnosis displayed high correlation [81]. Particularly, the benefits of upper endoscopy to conform atrophic change of gastric mucosa are generally accepted [82, 83]. Endoscopic feature of AG is the visibility of a vascular pattern of gastric mucosa (Fig. 18.3c) than normal gastric mucosa (Fig. 18.3a). Endoscopic finding of gastric IM (Fig. 18.3d) is defined as the replacement of the surface, foveolar, and glandular epithelium in the oxyntic or antral mucosa by intestinal epithelium. Actually, endoscopic finding of IM is observed as a mucosal nodular pattern, usually occurring after the occurrence of the AG. It is not difficult to diagnose severe cases of AG and IM properly by endoscopic findings, but it is difficult to make the diagnoses of mild AG and IM [80]. Sometimes endoscopic diagnosis is not correlated to histological diagnosis. Therefore, it is proper to biopsy in suspected cases of AG and IM. However, as atrophic mucosal changes are not distributed similarly in the whole gastric mucosa, multiple endoscopic biopsy of gastric mucosa does not always represent AG. Furthermore, it is difficult to take multiple biopsies of all subjects with simple gastritis [84, 85]. To resolve this clinical difficulty, noninvasive tests for precursor lesions such as serum level of pepsinogen (PG), gastrin-17, and *H. pylori* immunoglobulin G antibodies could be used as biomarkers of AG and IM to replace endoscopic biopsy [86]. Actually, the endoscopic, histological, and serological AG showed a relatively good correlation in the 2558 Korean subjects [87] (Fig. 18.7). However, as these three methods had a limitation, a multifactorial assessment might be needed to ameliorate the diagnostic accuracy of AG [87]. From this background, we will discuss about three diagnostic methods for AG and IM: endoscopic, histological, and PG I/II ratio in this chapter.

18.6.1 Endoscopic Diagnosis

Endoscopic diagnosis of AG and IM is the basic method. However, there is the possibility of low sensitivity, specificity, and interobserver variation. Endoscopic diagnosis of AG is made when the visibility of a vascular pattern followed by loss of gastric mucosal gland is present (Fig. 18.3c). There are various criteria to endoscopic classification of AG. AG due to *H. pylori* infection is more common in the East, and atrophic mucosal change progresses from the antrum to the corpus along with the lesser curvature (Fig. 18.8c); these endoscopic features reflect Kimura-Takemoto classification well [88]. The boundaries of the mucosal atrophy are called the F line (Fig. 18.8b), and AG is classified into six types. The closed-type AG indicates that the atrophic border remains on the lesser curvature, while the open-type AG means that the atrophic border no longer exists on the lesser curvature but extends along the anterior and posterior walls of

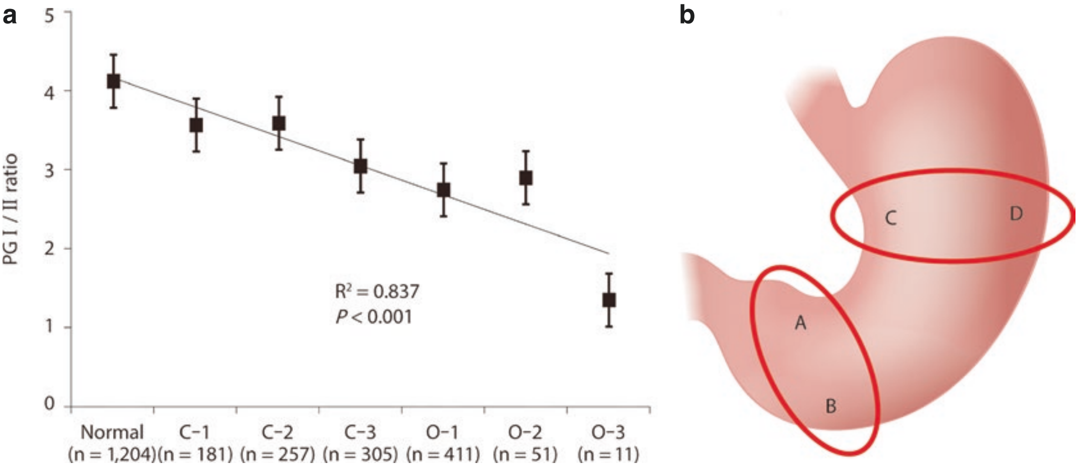


Fig. 18.7 Correlation between serum pepsinogen (PG) I/II ratio and endoscopic atrophic gastritis and gastric biopsy sites in the antrum and corpus. (**a**) The serum PG I/II ratio decreased significantly as gastric mucosal atrophy progressed. Data are presented as mean ± SE. (**b**) The gastric biopsy was taken from the lesser curvature (**A**) and greater curvature in the antrum (**B**) and from the lesser curvature (**C**) and greater curvature in the corpus (**D**) (Adapted from Lee et al. [87])

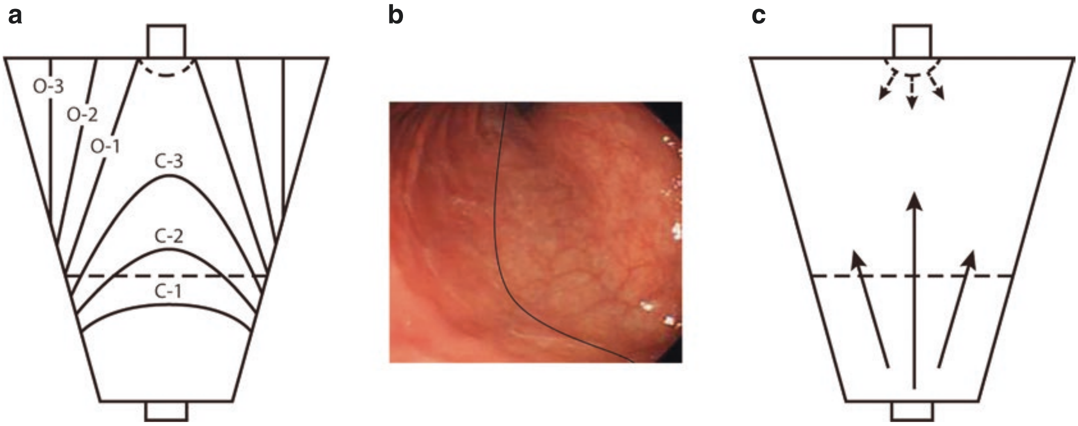


Fig. 18.8 Kimura-Takemoto classification of chronic atrophic gastritis: (**a**) atrophic gastritis (AG) is classified into six types. The closed-type AG indicates that the atrophic border remains on the lesser curvature of the stomach, while the open-type AG means that the atrophic border no longer exists on the lesser curvature but extends along the anterior and posterior walls of the stomach, (**b**) the boundaries of the mucosal atrophy is called as the F line, (**c**) progression of atrophic mucosal change from antrum to the corpus along with lesser curvature (Adapted from Kimura and Takemoto [82])

the stomach (Fig. 18.8a). However, Western physicians lack a good understanding of Kimura-Takemoto classification because the ratio of autoimmune gastritis is greater in the West than in the East and *H. pylori* infection rate is rather low [89]. In addition, the interobserver reliability is rather low in Kimura-Takemoto classification from endoscopic features. Other limitation of the endoscopic diagnosis of AG and IM is the lower sensitivity and specificity than the pathological diagnosis. In the study of 1333 subjects in South Korea, the sensitivity of the prevalence of endoscopic diagnosis of atrophy was significantly low in the age below 50 years old [80]. The sensitivity and specificity of endoscopy for the diagnosis of gastric atrophy based on histological findings were 61.5% and 57.7% in the antrum and were 46.8% and 76.4% in the corpus of the stomach [80]. Especially, endoscopic diagnosis of AG was inaccurate below 50 years of age because mild atrophic mucosal change may look normal in endoscopy [80]. In addition, the sensitivity and specificity of endoscopic IM diagnosis were also low in study by Lim et al. [90]. The sensitivity and specificity of endoscopic diagnosis of IM based on histology were 24.0% and 91.9% for the antrum and were 24.2% and 88.0% for the corpus. As indicated by a multivariate analysis, the activity of mucosal inflammation and the presence of endoscopic AG were associated with the sensitivity of endoscopic diagnosis of IM in the antrum, while benign gastric ulcers, dysplasia, and the presence of endoscopic AG were associated with the sensitivity of endoscopic diagnosis of IM in the corpus [90]. Thus, a high level of suspicion is important to increase the sensitivity of endoscopic diagnoses of IM, especially when adenoma, endoscopic AG, and ulcer are present, and confirmation of the doubt by histological diagnosis is necessary.

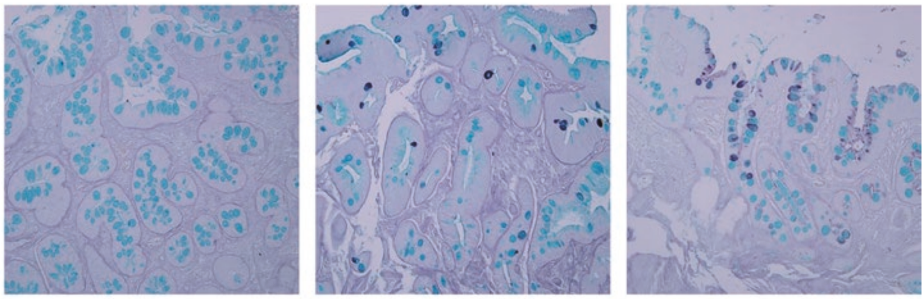
Other methods for endoscopic diagnosis of AG or IM are magnification chromoendoscopy and narrow band imaging (NBI). Several studies have suggested that chromoendoscopy with magnification could help to identify lesions of IM and dysplasia [91]. However, high-resolution magnifying endoscopy without chromoendoscopy also appears superior to standard endoscopy, allowing great accuracy for the diagnosis of *H. pylori* gastritis, IM, and dysplasia [92, 93]. The recent technology of NBI has been found to have good sensitivity and specificity for the diagnosis of gastric precancerous lesions [94, 95]. However, there is no agreement on which NBI patterns are associated with gastric precancerous lesions. So, further study is needed to apply NBI in diagnosis of AG and IM.

18.6.2 Histological Diagnosis

The second method of diagnosis of AG is histopathology . The updated Sydney system is the most widely accepted for classification and grading of gastritis. The updated Sydney system recommended five biopsies, two from the antrum, one from the incisura, and two from the corpus, because atrophic mucosal change and IM of gastric mucosa progress from antrum to corpus [67] (Fig. 18.4). If it is difficult to obtain at these three points, biopsies from the antrum and corpus are recommended. It is desirable to examine specimens from the antrum and corpus of the lesser and greater curvature, yet there are practical difficulties. Clinically, one biopsy specimen from the lesser curvature and the other from greater curvature are used to evaluate AG and IM [78, 87, 90].

Histological diagnosis is difficult when the specimens are inadequate. Deep portion of normal gastric mucosa is composed of different types of cells in the antrum and in the corpus. Normal gastric mucosa of the antrum is composed of mucous glands which secrete gastric mucus, and that of the corpus is composed of parietal cell and chief cell which secrete gastric acid and digestive enzymes. Sometimes severe inflammation obscures the gland's population, making it impossible to assess mucosal atrophy reliably. Such cases can be labeled as "indefinite for atrophy," and the final judgment can be deferred until the inflammation has regressed [96]. In addition, the diagnosis of "indefinite for atrophy" may be controversial among pathologists due to the difficulty in histological diagnosis [96].

To investigate the meaning of "indefinite for atrophy," our team evaluated clinical characteristics of cases reported as indefinite for atrophy in 3192 gastric biopsies obtained at a single medical center between 2003 and 2017 [97]. Cases categorized as indefinite atrophy (n = 1292) were compared with three groups, the absent atrophy group (n = 755), OLGA I–II group (n = 925), and OLGA III–IV group (n = 220), by considering age, sex, smoking, alcohol drinking, salty and spicy food diet, ABO blood type, family history of GC, and *H. pylori* infection that are known AG-associated risk factors [97]. Subjects aged ≥65 years (34.9% vs. 26.4%; $p < 0.001$), male (58.9% vs. 49.1%; $p < 0.001$), and *H. pylori*-infected subjects (82.5% vs. 63.3%; $p < 0.001$) were significantly more likely to be categorized



	Type I		Type II		Type III	
	Sialomucin	Sulfomucin	Sialomucin	Sulfomucin	Sialomucin	Sulfomucin
Goblet cell	+	−	+	±	+	+
Columnar cell	−	−	+	−	±	+

Fig. 18.9 Phenotype of intestinal metaplasia (IM) classified by mucin: type I IM expresses only sialomucins (*bright blue*), and types II and III express sialomucins (*bright blue*) and sulfomucins (*black*) (Adapted from Kang et al. [75])

into the indefinite atrophy group than absent atrophy group [97]. Subjects with family history of GC (21.9% vs. 25.7%, $p = 0.031$) and positive *H. pylori* infection (82.5% vs. 86.2%; $p = 0.019$) were significantly less likely to be in the indefinite atrophy group than low-risk OLGA group [97]. Subjects aged ≥65 years (52.3% vs. 34.9%; $p < 0.001$) and current/past smoker (70.0% vs. 50.9%; $p < 0.001$) were significantly more likely to be in the high-risk OLGA group than indefinite atrophy group [97]. These results could be interpretated that the pathological report of "indefinite for atrophy" suggests a higher plausibility of gastric atrophy than nonassessment due to a simple technical error [97].

IM is defined as the replacement of the surface, foveolar, and glandular epithelium in the oxyntic or antral mucosa by intestinal epithelium. These IM has been subtyped by classification of mucin expression. There are several classification systems for IM. The most widely used and useful classification was made by Jass and Filipe in 1981 [98]. Type I IM (complete) is positive for the sialomucin, and type II IM (incomplete) is positive for sialomucin in goblet and columnar cell, while type III IM (incomplete) is positive for sulfomucin [98]. Complete-type IM is similar to the small intestine in pathologic features and tests positive for the MUC2 secreted by goblet cells, while incomplete-type IM is similar to the large intestine in pathologic features and positive for MUC5AC and MUC6 secreted by the gastric mucosa and MUC2 [75, 99]. In addition, high-iron diamine-alcian blue (HID-AB) stain can help classify the types of IM. Type I IM expresses only sialomucins (bright blue) (Fig. 18.9, type I), and types II and III express sialomucins (bright blue) and sulfomucins (black) [75] (Fig. 18.9, type II and type III). Jass and Filipe reported that type III IM was associated with intestinal-type GC [98]. Other researchers have suggested that incomplete-type IM is associated with GC. As such, it is still controversial discussing which type of IM is related with gastric carcinogenesis [72, 73, 75]. In a cohort study of 861 subjects conducted in South Korea, type III IM was associated with aging ($p = 0.036$), and type II IM was associated with gastric carcinogenesis in the presence of *H. pylori* infection ($p < 0.05$) [75].

18.6.3 Diagnosis by Serum Pepsinogen I/II Ratio

The third diagnostic method of AG or IM is a measurement of serum PG I, II, and I/II ratio. The radioimmunoassay method was used to measure serum PG levels. Recently, a new method has emerged to measure PG level [100, 101]. The present methods to measure PG level are latex-enhanced turbidimetric immunoassay (HBi Corp, Seoul, Korea, imported from Shima Laboratories, Tokyo, Japan) [95, 100, 101] and enzyme-linked immunosorbent assays (Biohit ELISA kit; Biohit, Helsinki, Finland) [102, 103]. PG has been used to screen AG, IM, and gastric adenocarcinoma for more than 20 years because of its noninvasiveness and cost-effectiveness. In Japan, PG screening for GC has been used to improve population compliance [104]. However, PG screening is not accepted as a generalized screening method of AG or IM worldwide because of low-positive predictive value in other countries [105].

PG I is produced by chief cells in the antrum and corpus, while PG II is produced by the chief cells and mucous neck cells of the whole gastric mucosa. When gastric atrophy develops, chief cells are replaced by pyloric glands, leading to a decrease in PG I level, while PG II levels are relatively unaffected, so a low PG I/II ratio reflects the severity of AG [106, 107]. In a prospective study of 5113 subjects in Japan, screening for GC with PG I <70 ng/mL and PG I/II ratio <3 as the cutoff points had the sensitivity and specificity of 84.6% and 73.5%, respectively [108]. Generally PG I/II ratio 3.0 or less has been widely accepted as a cutoff value in several studies [108, 109]. However, in a recent Korean study which evaluated the relationship between PG level and AG, a new cutoff value of PG I/II ratio, 3.2, was suggested to diagnose AG [87]. This study suggested that serum PG I/II ratio remarkably decreased in correlation with the extent of atrophy by the Kimura-Takemoto classification [87]. In another Korean research, PG I and PG II were negatively correlated with AG, and only PG I/II ratio was positively correlated with AG [101]. Serum PG I and PG II were higher in *H. pylori*-positive than in *H. pylori*-negative subjects, because a growth rate of PG II was higher than that of PG I in *H. pylori*-positive individuals [87, 100, 101]. Moreover, a significantly positive correlation was found between age and the PG II ($p < 0.001$) and a negative correlation between age and the PG I/II ratio ($p < 0.001$) but no correlation between age and the PG I [101]. In addition, males had significantly higher PG I levels compared with females ($p < 0.001$) and had a slightly higher PG II levels without statistical significance, suggesting that the difference in the PG I levels observed between sex could be related to hormonal effects [101]. Consequently these results show that multiple factors could change the serum PG level. Thus, the proper cutoff value of PG level should be established in each country in order to increase sensitivity and specificity [87, 101].

18.7 Management for Atrophic Gastritis and Intestinal Metaplasia

Up to the present time, there are no unified clinical guidelines for prevention of GC regarding the classification of high-risk groups progressing to GC [110]. However, through many studies, AG and IM are considered as precancerous lesions. Prevention and treatment of AG and IG could decrease the prevalence of GC, requiring strategies to manage AG and IM [111]. As *H. pylori* infection triggers a multistep progression from chronic gastritis, AG, IM, and finally to invasive GC [31], *H. pylori* eradication is a key step in management strategy for AG and IM [112] even there are still arguments regarding the efficacy of *H. pylori* eradication, especially in case IM [9]. Another strategy is the surveillance to detect early gastric cancer (EGC) in the subjects with AG or IM, with additional managements of several risk factors of AG and IM [111]. Adequate consumption of vegetables and fruits seems to reduce the risk of cancer and decrease the incidence of GC in the West. Although some studies for premalignant gastric lesions have shown positive results of vitamin C, folic acid, and β-carotene supplementation, these results were not confirmed in a large meta-analysis [113–116]. However, in our study, the significant decrease in the prevalence and grades of atrophy and IM in the corpus of females despite decreased *H. pylori* infection, regardless of sex, suggests that sex- or gender-specific characteristics, such as alcohol, smoking, and eating habits, including salty or spicy foods, are related to the development of atrophy/IM in addition to *H. pylori* infection [117]. In the study of dietary intake of phytonutrients in relation to fruit and vegetable consumption in Korea, a higher proportion of women than men met the recommended intake of fruits and vegetables [118]. In addition, the Korean national health and nutrition examination surveys among three groups, men, menopausal women, and post-

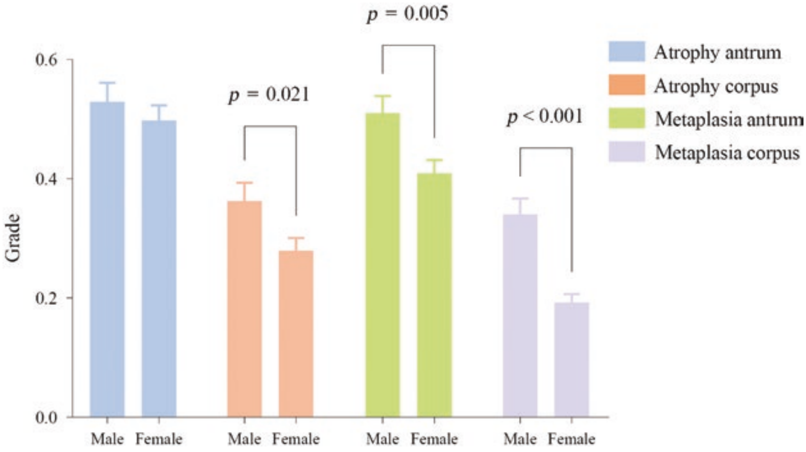


Fig. 18.10 Differences in the mean grades of atrophy and intestinal metaplasia (IM) between males and females. The mean grades of atrophy in the antrum, IM in the antrum, atrophy and IM in the corpus were significantly lower in females than in males analysis (Adapted from Kwon et al. [47])

menopausal women, showed that men's dietary carotenoid intake was the lowest [117]. Since fruits and vegetables are the main sources of dietary carotenoids, this study was consistent with the above findings that men consume less dietary fruits and vegetables than women. This difference in diet might explain the results in our study, with a significant decrease in prevalence and grades of atrophy/IM in the corpus only in females [47]. In addition to this sex difference in diet, there may be a sex difference in the severity of atrophy/IM. It is generally understood that the prevalence of *H. pylori* infection and gastric disorders in males is more common than that in females [46]. Similarly, the mean grades of atrophy and IM of females were different from those of males. That is, the mean grades of atrophy in the antrum, IM in the antrum, and IM in the corpus were significantly lower in females than in males ($p = 0.021$, $p = 0.005$, $p < 0.001$, respectively) [47] (Fig. 18.10). These lower grades of atrophy/IM in females might have caused a declining time trend in females in addition to healthier eating habits.

Taken together *H. pylori* eradication could be the main strategy for prevention of GC in individuals with AG and IM, but it may be helpful to change diet and supply vitamin C, folic acid, and β-carotene. Consequently, further large cohort study is needed to establish the optimal strategy of GC prevention in the presence of AG and IM.

18.8 Conclusions

To reduce the prevalence of GC, it is very important to classify and manage the high-risk groups of GC. Furthermore, *H. pylori* infection triggers a multistep inflammation from chronic gastritis, AG, IM, and finally to GC. Many studies have proved that AG and IM are precancerous lesions of GC and *H. pylori* infection is the most important cause of AG and IM. Diagnostic methods of AG and IM are endoscopic, histological findings, and PG I/II ratio. Each method has advantages and disadvantages, and it is necessary to select the proper test according to the individual cases. As the infection of *H. pylori* is the most important risk factor of AG and IM, it is important to perform *H. pylori* eradication to prevent the progression to GC. The appropriate time to prevent GC by *H. pylori* eradication is still under discussion. Controlling other risk factors such as diet, vitamin C, β-carotene, and folic acid may be useful to decrease the prevalence of GC.

References

1. Shiotani A, Cen P, Graham DY. Eradication of gastric cancer is now both possible and practical. Semin Cancer Biol. 2013;23:492–501.
2. Graham DY. *Campylobacter pylori* and peptic ulcer disease. Gastroenterology. 1989;96:615–25.

3. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, et al. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med. 2001;345:784–9.
4. Kamada T, Haruma K, Ito M, Inoue K, Manabe N, Matsumoto H, et al. Time trends in *Helicobacter pylori* infection and atrophic gastritis over 40 years in Japan. Helicobacter. 2014;20:192–8.
5. Graham DY. History of *Helicobacter pylori*, duodenal ulcer, gastric ulcer and gastric cancer. World J Gastroenterol. 2014;20:5191–204.
6. Tsai YC, Hsiao WH, Yang HB, Cheng HC, Chang WL, Lu CC, et al. The corpus-predominant gastritis index may serve as an early marker of *Helicobacter pylori*-infected patients at risk of gastric cancer. Aliment Pharmacol Ther. 2013;37:969–78.
7. Rugge M, Fassan M, Pizzi M, Farinati F, Sturniolo GC, Plebani M, Graham DY. Operative link for gastritis assessment vs operative link on intestinal metaplasia assessment. World J Gastroenterol. 2011;17:4596–601.
8. Wang J, Xu L, Shi R, Huang X, Li SW, Huang Z, et al. Gastric atrophy and intestinal metaplasia before and after *Helicobacter pylori* eradication: a meta-analysis. Digestion. 2011;83:253–60.
9. Kong YJ, Yi HG, Dai JC, Wei MX. Histological changes of gastric mucosa after *Helicobacter pylori* eradication: a systematic review and meta-analysis. World J Gastroenterol. 2014;20:5903–11.
10. Adamu MA, Weck MN, Gao L, Brenner H. Incidence of chronic atrophic gastritis: systematic review and meta-analysis of follow-up studies. Eur J Epidemiol. 2010;25:439–48.
11. Kim N, Park YS, Cho SI, Lee HS, Choe G, Kim IW, et al. Prevalence and risk factors of atrophic gastritis and intestinal metaplasia in a Korean population without significant gastroduodenal disease. Helicobacter. 2008;13:245–55.
12. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357:539–45.
13. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420:860–7.
14. Ohshima H, Tazawa H, Sylla BS, Sawa T. Prevention of human cancer by modulation of chronic inflammatory processes. Mutat Res. 2005;591:110–22.
15. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process—first American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res. 1992;52:6735–40.
16. Correa P. Chronic gastritis: a clinico-pathological classification. Am J Gastroenterol. 1988;83:504–9.
17. de Vries AC, Kuipers EJ. Epidemiology of premalignant gastric lesions: implications for the development of screening and surveillance strategies. Helicobacter. 2007;12 (Suppl 2):22–31.
18. Ohata H, Kitauchi S, Yoshimura N, Mugitani K, Iwane M, Nakamura H, et al. Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer. Int J Cancer. 2004;109:138–43.
19. Kim N, Park RY, Cho SI, Lim SH, Lee KH, Lee W, et al. *Helicobacter pylori* infection and development of gastric cancer in Korea: long-term follow-up. J Clin Gastroenterol. 2008;42:448–54.
20. Correa P, Haenszel W, Cuello C, Tannenbaum S, Archer M. A model for gastric cancer epidemiology. Lancet. 1975;2:58–60.
21. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand. 1965;64:31–49.
22. Gore RM. Gastric cancer. Clinical and pathologic features. Radiol Clin North Am. 1997;35:295–310.
23. Meining A, Morgner A, Miehlke S, Bayerdorffer E, Stolte M. Atrophy-metaplasia-dysplasia-carcinoma sequence in the stomach: a reality or merely an hypothesis? Best Pract Res Clin Gastroenterol. 2001;15:983–8.
24. Handa Y, Saitoh T, Kawaguchi M, Misaka R, Ohno H, Tsai CR, et al. Association of *Helicobacter pylori* and diffuse type gastric cancer. J Gastroenterol. 1996;31 (Suppl 9):29–32.
25. Parsonnet J, Vandersteen D, Goates J, Sibley RK, Pritikin J, Chang Y. *Helicobacter pylori* infection in intestinal- and diffuse-type gastric adenocarcinomas. J Natl Cancer Inst. 1991;83:640–3.
26. Yun CY, Kim N, Lee J, Lee JY, Hwang YJ, Lee HS, et al. Usefulness of OLGA and OLGIM system not only for intestinal type but also for diffuse type of gastric cancer, and no interaction among the gastric cancer risk factors. Helicobacter. 2018:e12542.
27. Tatsuta M, Iishi H, Nakaizumi A, Okuda S, Taniguchi H, Hiyama T, et al. Fundal atrophic gastritis as a risk factor for gastric cancer. Int J Cancer. 1993;53:70–4.
28. Leung WK, Sung JJ. Review article: intestinal metaplasia and gastric carcinogenesis. Aliment Pharmacol Ther. 2002;16:1209–16.
29. Chen CC, Liou JM, Lee YC, Hong TC, El-Omar EM, Wu MS. The interplay between *H. pylori* and gastrointestinal microbiota. Gut Microbes. 2021;13:e1909459.
30. Kato S, Matsukura N, Tsukada K, Matsuda N, Mizoshita T, Tsukamoto T, et al. *Helicobacter pylori* infection-negative gastric cancer in Japanese hospital patients: incidence and pathological characteristics. Cancer Sci. 2007;98:790–4.
31. Siurala M, Varis K, Wiljasalo M. Studies of patients with atrophic gastritis: a 10-15-year follow-up. Scand J Gastroenterol. 1966;1:40–8.

32. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984;1:1311–5.
33. Schistosomes, liver flukes and *Helicobacter pylori*. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7–14 June 1994. IARC Monogr Eval Carcinog Risks Hum. 1994;61:1–241.
34. Huang JQ, Sridhar S, Chen Y, Hunt RH. Meta-analysis of the relationship between *Helicobacter pylori* seropositivity and gastric cancer. Gastroenterology. 1998;114:1169–79.
35. Eslick GD, Lim LL, Byles JE, Xia HH, Talley NJ. Association of *Helicobacter pylori* infection with gastric carcinoma: a meta-analysis. Am J Gastroenterol. 1999;94:2373–9.
36. Rugge M. Gastric cancer risk in patients with *Helicobacter pylori* infection and following its eradication. Gastroenterol Clin North Am. 2015;44:609–24.
37. Wroblewski LE, Peek RM Jr, Wilson KT. *Helicobacter pylori* and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev. 2010;23:713–39.
38. Tsuji S, Kawai N, Tsujii M, Kawano S, Hori M. Review article: inflammation-related promotion of gastrointestinal carcinogenesis—a perigenetic pathway. Aliment Pharmacol Ther. 2003;18 (Suppl 1):82–9.
39. Suganuma M, Yamaguchi K, Ono Y, Matsumoto H, Hayashi T, Ogawa T, et al. TNF-alpha-inducing protein, a carcinogenic factor secreted from *H. pylori*, enters gastric cancer cells. Int J Cancer. 2008;123:117–22.
40. Kuipers EJ, Lundell L, Klinkenberg-Knol EC, Havu N, Festen HP, Liedman B, et al. Atrophic gastritis and *Helicobacter pylori* infection in patients with reflux esophagitis treated with omeprazole or fundoplication. N Engl J Med. 1996;334:1018–22.
41. Tepes B, Kavcic B, Zaletel LK, Gubina M, Ihan A, Poljak M, et al. Two- to four-year histological follow-up of gastric mucosa after *Helicobacter pylori* eradication. J Pathol. 1999;188:24–9.
42. Jonsson KA, Strom M, Bodemar G, Norrby K. Histologic changes in the gastroduodenal mucosa after long-term medical treatment with cimetidine or parietal cell vagotomy in patients with juxtapyloric ulcer disease. Scand J Gastroenterol. 1988;23:433–41.
43. Craanen ME, Dekker W, Blok P, Ferwerda J, Tytgat GN. Intestinal metaplasia and *Helicobacter pylori*: an endoscopic bioptic study of the gastric antrum. Gut. 1992;33:16–20.
44. Kim HJ, Choi BY, Byun TJ, Eun CS, Song KS, Kim YS, et al. The prevalence of atrophic gastritis and intestinal metaplasia according to gender, age and *Helicobacter pylori* infection in a rural population. J Prev Med Public Health. 2008;41:373–9.
45. Park HK, Kim N, Lee SW, Park JJ, Kim JI, Lee SY, et al. The distribution of endoscopic gastritis in 25,536 heath check-up subjects in Korea. J Helicobacter Upper Gastroint Res. 2012;12:171–7.
46. Joo YE, Park HK, Myung DS, Baik GH, Shin JE, Seo GS, et al. Prevalence and risk factors of atrophic gastritis and intestinal metaplasia: a nationwide multicenter prospective study in Korea. Gut Liver. 2013;7:303–10.
47. Kwon YJ, Kim N, Baek SM, Lee HS, Hwang YJ, Lee J, et al. Prevalence of histologic atrophic gastritis and intestinal metaplasia in the corpus has decreased over 15 years in females in the Korean population. Helicobacter. 2019;24:e12579.
48. Weck MN, Stegmaier C, Rothenbacher D, Brenner H. Epidemiology of chronic atrophic gastritis: population-based study among 9444 older adults from Germany. Aliment Pharmacol Ther. 2007;26:879–87.
49. Borch K, Jonsson KA, Petersson F, Redeen S, Mardh S, Franzen LE. Prevalence of gastroduodenitis and *Helicobacter pylori* infection in a general population sample: relations to symptomatology and life-style. Dig Dis Sci. 2000;45:1322–9.
50. Asaka M, Sugiyama T, Nobuta A, Kato M, Takeda H, Graham DY. Atrophic gastritis and intestinal metaplasia in Japan: results of a large multicenter study. Helicobacter. 2001;6:294–9.
51. Zou D, He J, Ma X, Liu W, Chen J, Shi X, et al. *Helicobacter pylori* infection and gastritis: the Systematic Investigation of gastrointestinaL diseases in China (SILC). J Gastroenterol Hepatol. 2011;26:908–15.
52. Eriksson NK, Karkkainen PA, Farkkila MA, Arkkila PE. Prevalence and distribution of gastric intestinal metaplasia and its subtypes. Dig Liver Dis. 2008;40:355–60.
53. Almouradi T, Hiatt T, Attar B. Gastric intestinal metaplasia in an underserved population in the USA: prevalence, epidemiologic and clinical features. Gastroenterol Res Pract. 2013;2013:856256.
54. Park YH, Kim N. Review of atrophic gastritis and intestinal metaplasia as a premalignant lesion of gastric cancer. J Cancer Prev. 2015;20:25–40.
55. Stemmermann GN, Mower H. Gastritis, nitrosamines, and gastric cancer. J Clin Gastroenterol. 1981;3:23–7.
56. Atherton JC. The clinical relevance of strain types of *Helicobacter pylori*. Gut. 1997;40:701–3.
57. Kuipers EJ, Perez-Perez GI, Meuwissen SG, Blaser MJ. *Helicobacter pylori* and atrophic gastritis: importance of the cagA status. J Natl Cancer Inst. 1995;87:1777–80.
58. Huang JQ, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-analysis of the relationship between cagA seropositivity and gastric cancer. Gastroenterology. 2003;125:1636–44.
59. Basso D, Zambon CF, Letley DP, Stranges A, Marchet A, Rhead JL, et al. Clinical relevance of *Helicobacter pylori* cagA and vacA gene polymorphisms. Gastroenterology. 2008;135:91–9.

60. Nogueira C, Figueiredo C, Carneiro F, Gomes AT, Barreira R, Figueira P, et al. *Helicobacter pylori* genotypes may determine gastric histopathology. Am J Pathol. 2001;158:647–54.
61. Yamaoka Y. Increasing evidence of the role of *Helicobacter pylori* SabA in the pathogenesis of gastroduodenal disease. J Infect Dev Ctries. 2008;2:174–81.
62. Yamaoka Y, Kodama T, Gutierrez O, Kim JG, Kashima K, Graham DY. Relationship between *Helicobacter pylori* iceA, cagA, and vacA status and clinical outcome: studies in four different countries. J Clin Microbiol. 1999;37:2274–9.
63. Van Doorn LJ, Figueiredo C, Megraud F, Pena S, Midolo P, Queiroz DM, et al. Geographic distribution of vacA allelic types of *Helicobacter pylori*. Gastroenterology. 1999;116:823–30.
64. Kersulyte D, Mukhopadhyay AK, Velapatino B, Su W, Pan Z, Garcia C, et al. Differences in genotypes of *Helicobacter pylori* from different human populations. J Bacteriol. 2000;182:3210–8.
65. Schindler R. Chronic gastritis. Bull N Y Acad Med. 1939;15:322–37.
66. Misiewicz JJ. The Sydney system: a new classification of gastritis. Introduction. J Gastroenterol Hepatol. 1991;6:207–8.
67. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International workshop on the histopathology of gastritis, Houston 1994. Am J Surg Pathol. 1996;20:1161–81.
68. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, et al. Kyoto global consensus report on *Helicobacter pylori* gastritis. Gut. 2015;64:1353–67.
69. Rugge M, Meggio A, Pennelli G, Piscioli F, Giacomelli L, De Pretis G, et al. Gastritis staging in clinical practice: the OLGA staging system. Gut. 2007;56:631–6.
70. Rugge M, de Boni M, Pennelli G, de Bona M, Giacomelli L, Fassan M, et al. Gastritis OLGA-staging and gastric cancer risk: a twelve-year clinico-pathological follow-up study. Aliment Pharmacol Ther. 2010;31:1104–11.
71. Correa P, Piazuelo MB, Wilson KT. Pathology of gastric intestinal metaplasia: clinical implications. Am J Gastroenterol. 2010;105:493–8.
72. Filipe MI, Munoz N, Matko I, Kato I, Pompe-Kirn V, Jutersek A, et al. Intestinal metaplasia types and the risk of gastric cancer: a cohort study in Slovenia. Int J Cancer. 1994;57:324–9.
73. Rokkas T, Filipe MI, Sladen GE. Detection of an increased incidence of early gastric cancer in patients with intestinal metaplasia type III who are closely followed up. Gut. 1991;32:1110–3.
74. Conchillo JM, Houben G, de Bruine A, Stockbrugger R. Is type III intestinal metaplasia an obligatory precancerous lesion in intestinal-type gastric carcinoma? Eur J Cancer Prev. 2001;10:307–12.
75. Kang KP, Lee HS, Kim N, Kang HM, Park YS, Lee DH, et al. Role of intestinal metaplasia subtyping in the risk of gastric cancer in Korea. J Gastroenterol Hepatol. 2009;24:140–8.
76. Dinis-Ribeiro M, Areia M, de Vries AC, Marcos-Pinto R, Monteiro-Soares M, O'Connor A, et al. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European *Helicobacter* Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). Endoscopy. 2012;44:74–94.
77. Gonzalez CA, Sanz-Anquela JM, Gisbert JP, Correa P. Utility of subtyping intestinal metaplasia as marker of gastric cancer risk. A review of the evidence. Int J Cancer. 2013;133:1023–32.
78. Jonsson KA, Gotthard R, Bodemar G, Brodin U. The clinical relevance of endoscopic and histologic inflammation of gastroduodenal mucosa in dyspepsia of unknown origin. Scand J Gastroenterol. 1989;24:385–95.
79. Redéen S, Petersson F, Jönsson KA, Borch K. Relationship of gastroscopic features to histological findings in gastritis and *Helicobacter pylori* infection in a general population sample. Endoscopy. 2003;35:946–50.
80. Eshmuratov A, Nah JC, Kim N, Lee HS, Lee HE, Lee BH, et al. The correlation of endoscopic and histological diagnosis of gastric atrophy. Dig Dis Sci. 2010;55:1364–75.
81. Fung WP, Papadimitriou JM, Matz LR. Endoscopic, histological and ultrastructural correlations in chronic gastritis. Am J Gastroenterol. 1979;71:269–79.
82. Kimura K, Takemoto T. An endoscopic recognition of the atrophic border and its significance in chronic gastritis. Endoscopy. 1969;1:87–97.
83. Liu Y, Uemura N, Xiao SD, Tytgat GN, Kate FJ. Agreement between endoscopic and histological gastric atrophy scores. J Gastroenterol. 2005;40:123–7.
84. Toukan AU, Kamal MF, Amr SS, Arnaout MA, Abu-Romiyeh AS. Gastroduodenal inflammation in patients with non-ulcer dyspepsia. A controlled endoscopic and morphometric study. Dig Dis Sci. 1985;30:313–20.
85. Eriksson NK, Farkkila MA, Voutilainen ME, Arkkila PE. The clinical value of taking routine biopsies from the incisura angularis during gastroscopy. Endoscopy. 2005;37:532–6.
86. Cao Q, Ran ZH, Xiao SD. Screening of atrophic gastritis and gastric cancer by serum pepsinogen, gastrin-17 and *Helicobacter pylori* immunoglobulin G antibodies. J Dig Dis. 2007;8:15–22.
87. Lee JY, Kim N, Lee HS, Oh JC, Kwon YH, Choi YJ, et al. Correlations among endoscopic, histologic and serologic diagnoses for the assessment of atrophic gastritis. J Cancer Prev. 2014;19:47–55.
88. Kimura K, Satoh K, Ido K, Taniguchi Y, Takimoto T, Takemoto T. Gastritis in the Japanese stomach. Scand J Gastroenterol Suppl. 1996;214:17–20. discussion 21-13.

89. Neumann WL, Coss E, Rugge M, Genta RM. Autoimmune atrophic gastritis—pathogenesis, pathology and management. Nat Rev Gastroenterol Hepatol. 2013;10:529–41.
90. Lim JH, Kim N, Lee HS, Choe G, Jo SY, Chon I, et al. Correlation between endoscopic and histological diagnoses of gastric intestinal metaplasia. Gut Liver. 2013;7:41–50.
91. Dinis-Ribeiro M, da Costa-Pereira A, Lopes C, Lara-Santos L, Guilherme M, Moreira-Dias L, et al. Magnification chromoendoscopy for the diagnosis of gastric intestinal metaplasia and dysplasia. Gastrointest Endosc. 2003;57:498–504.
92. Anagnostopoulos GK, Yao K, Kaye P, Fogden E, Fortun P, Shonde A, et al. High-resolution magnification endoscopy can reliably identify normal gastric mucosa, *Helicobacter pylori*-associated gastritis, and gastric atrophy. Endoscopy. 2007;39:202–7.
93. Gonen C, Simsek I, Sarioglu S, Akpinar H. Comparison of high resolution magnifying endoscopy and standard videoendoscopy for the diagnosis of *Helicobacter pylori* gastritis in routine clinical practice: a prospective study. Helicobacter. 2009;14:12–21.
94. Kadowaki S, Tanaka K, Toyoda H, Kosaka R, Imoto I, Hamada Y, et al. Ease of early gastric cancer demarcation recognition: a comparison of four magnifying endoscopy methods. J Gastroenterol Hepatol. 2009;24:1625–30.
95. Capelle LG, Haringsma J, de Vries AC, Steyerberg EW, Biermann K, van Dekken H, et al. Narrow band imaging for the detection of gastric intestinal metaplasia and dysplasia during surveillance endoscopy. Dig Dis Sci. 2010;55:3442–8.
96. Rugge M, Correa P, Dixon MF, Fiocca R, Hattori T, Lechago J, et al. Gastric mucosal atrophy: interobserver consistency using new criteria for classification and grading. Aliment Pharmacol Ther. 2002;16:1249–59.
97. Kim HJ, Kim N, Yun CY, Lee HS. The clinical meaning of the "indefinite for atrophy" lesions within gastric mucosa biopsy specimens in a region with a high prevalence of gastric cancer. Helicobacter. 2019;24:e12605.
98. Jass JR, Filipe MI. The mucin profiles of normal gastric mucosa, intestinal metaplasia and its variants and gastric carcinoma. Histochem J. 1981;13:931–9.
99. Kang HM, Kim N, Park YS, Hwang JH, Kim JW, Jeong SH, et al. Effects of *Helicobacter pylori* infection on gastric mucin expression. J Clin Gastroenterol. 2008;42:29–35.
100. Kang JM, Kim N, Yoo JY, Park YS, Lee DH, Kim HY, et al. The role of serum pepsinogen and gastrin test for the detection of gastric cancer in Korea. Helicobacter. 2008;13:146–56.
101. Kim HY, Kim N, Kang JM, Park YS, Lee DH, Kim YR, et al. Clinical meaning of pepsinogen test and *Helicobacter pylori* serology in the health check-up population in Korea. Eur J Gastroenterol Hepatol. 2009;21:606–12.
102. Ren JS, Kamangar F, Qiao YL, Taylor PR, Liang H, Dawsey SM, et al. Serum pepsinogens and risk of gastric and oesophageal cancers in the general population nutrition intervention trail cohort. Gut. 2009;58:636–42.
103. Iijima K, Abe Y, Kikuchi R, Koike T, Ohara S, Sipponen P, Shimosegawa T. Serum biomarker tests are useful in delineating between patients with gastric atrophy and normal, healthy stomach. World J Gastroenterol. 2009;15:853–9.
104. Miki K, Fujishiro M, Kodashima S, Yahagi N. Long-term results of gastric cancer screening using the serum pepsinogen test method among an asymptomatic middle-aged Japanese population. Dig Endosc. 2009;21:78–81.
105. Ley C, Mohar A, Guarner J, Herrera-Goepfert R, Figueroa LS, Halperin D, et al. Screening markers for chronic atrophic gastritis in Chiapas. Mex Cancer Epidemiol Biomark Prev. 2001;10:107–12.
106. Miki K, Ichinose M, Kawamura N, Matsushima M, Ahmad HB, Kimura M, et al. The significance of low serum pepsinogen levels to detect stomach cancer associated with extensive chronic gastritis in Japanese subjects. Jpn J Cancer Res. 1989;80:111–4.
107. Samloff IM, Varis K, Ihamaki T, Siurala M, Rotter JI. Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. Gastroenterology. 1982;83:204–9.
108. Yanaoka K, Oka M, Mukoubayashi C, Yoshimura N, Enomoto S, Iguchi M, et al. Cancer high-risk subjects identified by serum pepsinogen tests: outcomes after 10-year follow-up in asymptomatic middle-aged males. Cancer Epidemiol Biomarkers Prev. 2008;17:838–45.
109. Dinis-Ribeiro M, Yamaki G, Miki K, Costa-Pereira A, Matsukawa M, Kurihara M. Meta-analysis on the validity of pepsinogen test for gastric carcinoma, dysplasia or chronic atrophic gastritis screening. J Med Screen. 2004;11:141–7.
110. Gonzalez CA, Agudo A. Carcinogenesis, prevention and early detection of gastric cancer: where we are and where we should go. Int J Cancer. 2012;130:745–53.
111. Yoon H, Kim N. Diagnosis and management of high risk groups of gastric cancer. Gut Liver. 2015;9:5–17.
112. Hwang YJ, Kim N, Lee HS, Lee JB, Choi YJ, Yoon H, et al. Reversibility of atrophic gastritis and intestinal metaplasia after *Helicobacter pylori* eradication—a prospective study for up to 10 years. Aliment Pharmacol Ther. 2018;47:380–90.
113. Correa P, Fontham ET, Bravo JC, Bravo LE, Ruiz B, Zarama G, et al. Chemoprevention of gastric dysplasia: randomized trial of antioxidant supplements and anti-*Helicobacter pylori* therapy. J Natl Cancer Inst. 2000;92:1881–8.
114. Cao DZ, Sun WH, Ou XL, Yu Q, Yu T, Zhang YZ, et al. Effects of folic acid on epithelial apoptosis and expression of Bcl-2 and p53 in premalignant gastric lesions. World J Gastroenterol. 2005;11:1571–6.

115. Zullo A, Rinaldi V, Hassan C, Diana F, Winn S, Castagna G, et al. Ascorbic acid and intestinal metaplasia in the stomach: a prospective, randomized study. Aliment Pharmacol Ther. 2000;14:1303–9.
116. Bjelakovic G, Nikolova D, Simonetti RG, Gluud C. Antioxidant supplements for prevention of gastrointestinal cancers: a systematic review and meta-analysis. Lancet. 2004;364:1219–28.
117. Regu GM, Kim H, Kim YJ, Paek JE, Lee G, Chang N, et al. Association between dietary carotenoid intake and bone mineral density in Korean adults aged 30–75 years using data from the fourth and fifth Korean national health and nutrition examination surveys (2008–2011). Nutrients. 2017;9:E1025.
118. Lee HS, Cho YH, Park J, Shin HR, Sung MK. Dietary intake of phytonutrients in relation to fruit and vegetable consumption in Korea. J Acad Nutr Diet. 2013;113:1194–7.

19 Functional Dyspepsia

Sung Eun Kim

Abstract

Functional dyspepsia (FD) is defined as a clinical condition in which pain or discomfort arises from the gastroduodenal area in the absence of any organic, systemic, or metabolic disease that could explain the symptoms. Dyspeptic symptoms must be present for the previous 3 months with symptom onset at least 6 months before diagnosis according to the Rome IV criteria. *Helicobacter pylori* (*H. pylori*) infection is considered a predisposing factor for FD, because *H. pylori* is known not only to induce pathologic change of the gastric mucosa but also to affect several gastrointestinal hormones. Furthermore, recent studies showed that *H. pylori* could influence molecular biology causing motility disorders. Unfortunately, the results are still controversial, and further studies are needed to evaluate the role of *H. pylori*. Even a few years ago, *H. pylori* eradication was suggested as one of the treatment options for FD; however recently, *H. pylori* eradication is being considered as a first-line treatment for FD. In countries with a high prevalence of *H. pylori* infection, such as Korea, *H. pylori* eradication might improve the symptoms of FD patients. Several meta-analyses reported that *H. pylori* eradication could improve symptoms in patients with FD compared to placebo; however, there are limitations, including heterogeneity of enrolled studies. Therefore, well-designed, prospective studies are required to confirm the effect of *H. pylori* eradication in FD.

Keywords

Disease eradication · Dyspepsia · *Helicobacter pylori*

19.1 Introduction

Dyspepsia is one of the most prevalent gastrointestinal (GI) diseases, and a pooled prevalence of dyspepsia is 21.8% worldwide according to a systematic review and meta-analysis study [1]. Patients with dyspeptic symptoms usually suffer from heartburn, epigastric pain, postprandial discomfort, bloating, and a heavy feeling in the upper abdominal area. These symptoms are generally chronic and can decrease quality of life. Some patients with dyspeptic symptoms are diagnosed with functional dyspepsia (FD), which is defined as chronic and recurrent gastroduodenal discomfort without evidence of organic or systemic disease, such as peptic ulcer, GI malignancy, gastroesophageal reflux disease, or pancreatobiliary disease. Of patients who were referred to the department of gastroenterology at

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a tertiary hospital in Korea, about 8–20% had organic diseases, and about 70–92% had functional gastrointestinal disorders (FGIDs). FD was the most common disorder among the patients with FGIDs [2]. According to the National Health Insurance Corporation database in Korea, FGIDs lead to a significant socioeconomic burden [3]. This situation is similar not only in Korea, but also all over the world. Patients with FD have experienced increased healthcare costs due to loss of productivity, which is verified in a cross-sectional population-based study [4].

Several factors have been suggested to induce the symptoms of FD, including disturbed gastroduodenal motility, visceral hypersensitivity, brain-gut interactions, duodenal low-grade mucosal inflammation, immune alteration, genetic susceptibility, and gut microbiota dysbiosis. Among them, the direct connection between *Helicobacter pylori* (*H. pylori*) infection and FD remains controversial. Given this background, the aims of this chapter are to evaluate the relationship between FD and *H. pylori* infection, to assess the role of *H. pylori* in FD pathogenesis, and to assess the effect of *H. pylori* eradiation on treatment of FD.

19.2 Definition of Functional Dyspepsia

Prior to investigating the relationship between *H. pylori* infection and FD, we reviewed the definition of FD in detail. According to the Rome IV criteria, FD is defined as the existence of symptoms seeming to originate in the gastroduodenal area but with a negative diagnostic workup for organic or metabolic diseases that would explain the pain, including upper endoscopy, abdomen ultrasonography, laboratory findings, computed tomography (CT), or other modalities [5]. As with the Rome III criteria, FD was divided into four symptoms under the Rome IV criteria as follows: bothersome epigastric pain, bothersome epigastric burning, bothersome postprandial fullness, and bothersome early satiation. These symptoms must have been present for the previous 3 months with symptom onset at least 6 months before diagnosis. In addition, they presented two subcategories of FD based on cohort and population-based studies: postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS) [6–8]. PDS must include one or both of the following symptoms: bothersome postprandial fullness severe enough to influence on usual activities after regular-sized meals at least 3 days per week and bothersome early satiety severe enough to prevent finishing a regular-sized meal at least 3 days per week. In addition, patients with PDS can experience bloating in the upper abdominal area, postprandial nausea, or belching. Furthermore, PDS may coexist with EPS. EPS includes bothersome epigastric pain or burning of severe enough to influence on usual activities at least 1 day per week. This epigastric pain or burning should be intermittent, should not be generalized or localized to other abdominal or chest areas, should not be alleviated by defecation or passage of flatus, and should not be associated with gallbladder or sphincter of Oddi disorders. Additionally, the pain is usually induced or resolved by intake of a meal but may also happen during a fasting state [5] (Table 19.1).

Table 19.1 Rome IV criteria for functional dyspepsia

Diagnostic criteria[a] for functional dyspepsia must include one or more of the following symptoms:
(a) Bothersome postprandial fullness
(b) Bothersome early satiety
(c) Bothersome epigastric pain
(d) Bothersome epigastric burning
With no evidence of structural disease that is likely to explain symptoms (including upper endoscopy)
1. Postprandial distress syndrome
Diagnostic criteria must include one or both of the following symptoms:
(a) Bothersome postprandial fullness of severe enough to influence on daily activities, at least 3 days per week
(b) Bothersome early satiety of severe enough to prevent finishing a regular meal, at least 3 days per week
Other supportive criteria:
(a) Upper abdominal bloating, nausea, and excessive belching can be present
(b) Epigastric pain syndrome may coexist
(c) Vomiting requires consideration of another disorder
(d) Heartburn may coexist
(e) Not relieved by defecation or passage of flatus
*Other functional gastrointestinal disorder (e.g., gastroesophageal reflux disease, irritable bowel syndrome) may coexist
2. Epigastric pain syndrome
Diagnostic criteria must include all of the following symptoms:
(a) Bothersome pain localized to the epigastrium of severe enough to influence on daily activities, at least 1 day per week
(b) And/or bothersome burning localized to the epigastrium of severe enough to influence on daily activities, at least 1 day per week
Other supportive criteria:
(a) The pain is commonly induced or relieved by ingestion of a meal but may occur while fasting
(b) Postprandial distress syndrome may coexist
(c) Vomiting requires consideration of another disorder
(d) Heartburn may coexist
(e) Not fulfilling criteria for gallbladder and sphincter of Oddi disorders
(f) Not relieved by defecation or passage of flatus
*Other functional gastrointestinal disorder (e.g., gastroesophageal reflux disease, irritable bowel syndrome) may coexist

Modified from Stanghellini et al. [5]

[a] Criteria must be fulfilled for the previous 3 months with symptom onset at least 6 months before diagnosis

19.3 Pathophysiology of Functional Dyspepsia

FD is a complex disease that can arise from a variety of causes. Unfortunately, as stated above, the association between FD and *H. pylori* infection has not been clearly established. Recently, several studies have suggested that the relationship between FD and *H. pylori* infection may play a role in FD pathophysiology.

Several studies have reported that some patients acquired irritable bowel syndrome (IBS) symptoms after a GI infection. Similarly, several studies showed that a subset of patients developed FD following an episode of GI infection [9, 10]. A meta-analysis reported that the odds ratio (OR) for the occurrence of postinfectious FD was 2.54 (95% confidence interval [CI], 1.76–3.65) more than 6 months after acute gastroenteritis compared with the control [11]. Another meta-analysis found an OR of 2.18 (95% CI, 1.70–2.81) for FD risk following acute gastroenteritis [12]. Taken together, GI infection is associated with an increased risk of FD, supporting an inflammatory and immunological mechanism in the pathogenesis of FD [13]. *H. pylori* infection is the main cause of gastroduodenal inflammation [14] and provokes activation of a complex cytokine and chemokine response in the gastric mucosa [15], which may induce dyspepsia [16].

H. pylori infection induces gastric acid hypersecretion in the gastric antral mucosa [17]. Antral-predominant gastritis occurs in about 10–15% of patients with *H. pylori* infection, likely due to gastric acid hypersecretion [18]. Patients with antral-predominant gastritis due to *H. pylori* infection showed decreased somatostatin release in the antral gland area, which leads to increased gastrin release with subsequent rise in acid secretion. This mechanism may explain dyspepsia.

In addition to clinical symptoms, *H. pylori* infection induces macroscopic and microscopic changes in the gastric mucosa that, along with lifestyle factors, may increase the risk of atrophic gastritis and intestinal metaplasia, leading to gastric cancer (GC) [19–21]. However, the results of studies that investigated the relationship between severity of histological gastritis and dyspepsia symptoms were varied. Czinn et al. [22] revealed an association between the severity of inflammation and epigastric pain. Likewise, another study group also revealed an indirect association between the severity of inflammation on the gastric corpus and the severity of symptoms [23]. However, there was no significant difference between the severity of dyspepsia symptoms and the severity of histological gastritis in other studies [24, 25]. Consequently, it is difficult to draw firm conclusions about the relationship between the severity of gastritis and the severity of dyspeptic symptoms, and further studies are needed to investigate this association.

Among the GI hormones studied so far, ghrelin and leptin are representative hormones controlling appetite. Ghrelin promotes appetite and food intake and stimulates gastric emptying and gastric acid secretion [26]. These functions are partially achieved via vagal nerve pathways [27]. Plasma ghrelin concentrations are elevated in gastroduodenal mucosal injury for the purpose of gastroduodenal cytoprotection [28, 29]. However, plasma ghrelin levels decrease in the severe gastric mucosal atrophic state induced by *H. pylori* infection [30, 31]. Thus, *H. pylori* infection may be related to induction of gastric motor dysfunction and reduction of appetite by suppression of ghrelin secretion [16]. Ghrelin may therefore play a role in the occurrence of FD, especially that related to *H. pylori*. Studies have revealed that patients with FD experience changing plasma ghrelin levels, which are frequently related to FD symptom scores [32, 33]. A few studies reported that plasma ghrelin levels were significantly decreased in dysmotility-like FD patients with postprandial fullness and/or early satiety [34, 35]. A genetic polymorphism study revealed that the Leu72Met (408C > A) single nucleotide polymorphism of the ghrelin gene was significantly related to early-phase gastric emptying in patients with FD [36]. Akamizu et al. [37] revealed that repeated ghrelin administration increased food intake in patients with FD; however, another study showed that there was no significant difference between patients with dysmotility-like FD and healthy controls [32]. Increased plasma ghrelin levels in patients with FD were also reported [38]. Regarding *H. pylori* eradication, an interesting study reported that *H. pylori* eradication affected ghrelin and symptoms of FD. Choi et al. [39] reported that plasma acyl ghrelin level was elevated and gastric preproghrelin mRNA expression was upregulated after 1 year of *H. pylori* eradication in patients with FD. In addition, PDS symptoms were attenuated in patients with elevated ghrelin level, therefore a contribution for ghrelin in PDS symptoms has been suggested. Leptin also activates vagal nerve terminals, decreases appetite, and enhances gastric mucin secretion [40]. Similar to ghrelin, leptin may induce the occurrence of FD. A study in Iran reported that serum leptin levels were higher in patients with dysmotility-like dyspepsia [41]. Moreover, *H. pylori*-infected patients had increased serum leptin concentrations and leptin mRNA expression in the gastric mucosa [41, 42], suggesting that *H. pylori* infection could cause decreased appetite by stimulating serum leptin secretion. Cholecystokinin is a GI hormone released by I-cell in the upper small intestine for the digestion of high fat or protein food intake. Basal levels of cholecystokinin were significantly lower in *H. pylori*-infected patients with dyspepsia than in *H. pylori*-negative patients [43]. Several studies conducted to determine the correlation of FD patients with GI peptide hormones. Unfortunately, there are only a few studies investigating the correlation of *H. pylori*-positive FD patients with GI hormones, such as ghrelin, leptin, or cholecystokinin [44]. Thus, the association between GI hormones and dyspepsia with *H. pylori* remains speculative.

Since the late 2000s, the role of duodenum for FD, including low-grade mucosal inflammation and immune alteration, has been proposed. After Talley et al. [45] found that number of duodenal eosinophils in patients with nonulcer dyspepsia, particularly those with early satiety, were significantly higher than in controls, several studies were conducted to demonstrate the relationship between FD and both duodenal eosinophil and mast cell counts, and their degranulation. When eosinophil counts in patients with FD and asymptomatic controls were investigated, the OR for FD patients with high eosinophil counts in the duodenal bulb and duodenal second portion were 11.7 (95% CI, 3.9–34.9) and 7.3 (95% CI, 2.9–18.1), respectively, compared with asymptomatic controls [45]. *H. pylori* infection induces eosinophil aggregation in the gastric mucosa [46]. Another study also reported that eosinophilia in the duodenal second portion was significantly increased in FD patients with postprandial fullness, early satiety, and abdominal pain [47]. A recent meta-analysis study including 22 case-control studies covering duodenal eosinophils and mast cells in FD was published [48]. Duodenal eosinophils (standardized mean difference [SMD] 1.29; 95% CI, 0.85–1.73; $p = 0.0001$) and mast cells (SMD 2.11; 95% CI, 1.14–3.07; $p = 0.0001$) were significantly higher FD patients compared with controls, and degranulation of duodenal eosinophils (OR 3.78; 95% CI, 6.76–4.48; $p = 0.0001$) was increased in FD patients compared with controls. In terms of *H. pylori* status, duodenal eosinophils were significantly increased in *H. pylori*-negative FD patients compared with controls (SMD 3.98; 95% CI, 2.13–5.84; $p = 0.0001$); however, no significant difference was shown in *H. pylori*-positive FD patients compared with *H. pylori*-positive controls (SMD 0.44; 95% CI, −0.51 to 1.39; $p = 0.364$). *H. pylori* infection may upregulate gastric and duodenal eosinophil counts in both FD patients and controls, and it is presumed that duodenal eosinophil counts do not show significant difference between *H. pylori*-positive FD patients and *H. pylori*-positive controls. Therefore, *H. pylori* infection might be influenced to duodenal eosinophilia alongside the occurrence of FD. Intraepithelial lymphocytes in patients with FD and healthy controls were also evaluated by a study in France [49]. The number of intraepithelial lymphocytes were significantly higher in *H. pylori*-positive FD patients compared to healthy controls, but there was no significant difference between *H. pylori*-negative FD patients and healthy controls. The expression of CD95/Fas and HLA-DR expressing CD3+ intraepithelial lymphocytes was significantly lower in *H. pylori*-negative FD patients than in healthy controls. These results suggest that the phenotypic characteristics of intraepithelial lymphocytes might explain the difference between *H. pylori*-positive FD and *H. pylori*-negative FD.

Microbiota are one of the most noteworthy academic fields in recent years, and researches on the association between *H. pylori* and gastric microbiota have been conducted. According to those findings, most studies reported that *H. pylori*-positive and *H. pylori*-negative groups had differences in microbiota. In the *H. pylori*-positive status, an increased abundance of Proteobacteria, Spirochetes, and Acidobacteria, and decreased abundance of Actinobacteria, Bacteroidetes, and Firmicutes were revealed compared with *H. pylori*-negative status [50]. Another study using 16S rRNA gene amplicon sequencing, found that *H. pylori*-positive subjects had significantly lower bacterial richness as well as the operational taxonomic unit counts and Shannon and Simpson indices compared with *H. pylori*-negative subjects [51]. In addition, *H. pylori*-negative gastritis was connected with enrichment of Firmicutes, Fusobacteria, Bacteroidetes, and Actinobacteria at phylum level.

As regards FD, few studies evaluated the association between FD and microbiota [52].

Because *H. pylori* infection affects the microbiota, most FD studies performed excluding *H. pylori*-positive patients. Zhong et al. [53] investigated duodenal mucosal microbiota in FD patients and controls. Although the number of patients and controls were small, *Streptococcus* was the predominant genus in both FD patients and control subjects. The relative abundance of the anaerobic genera *Prevotella, Veillonella,* and *Actinomyces* was significantly decreased in patients with FD. Furthermore, a negative correlation between duodenal mucosal bacterial load and quality of life was identified, while as bacterial load increased, bacterial diversity decreased. A notable study on FD and duodenal mucosa-associated microbiota, which enrolled 86 subjects (56 FD, 30 controls), was reported recently. The relative abundances of predominant phyla were Firmicutes, Bacteroidota, and Fusobacteriota, and those were linked to symptom burden in FD [54]. The relative abundances of *Streptococcus* and *Prevotella* were negatively correlated in both FD and control subjects. Strikingly, the relative abundance of *Veillonella* genus was inversely correlated with gastric emptying time in both FD and control subjects [54]. Therefore, the study suggested the possibility that a duodenal mucosa-associated microbiota might be related to FD symptoms and gastric emptying.

Taken together, *H. pylori* is presumed to be contributing to the development of FD. Several studies suggested that *H. pylori*-positive dyspepsia, or *H. pylori*-associated dyspepsia, is not a functional disorder but an organic disease with many and various mechanisms of pathophysiology. Therefore, some study groups proposed that *H. pylori*-associated dyspepsia is different from FD and should be separated [16, 19]. Kyoto global consensus report and the Japanese Society of Gastroenterology (JSGE) guidelines were clarified that *H. pylori* infection is the cause of dyspepsia in a subset of patients, and *H. pylori*-associated dyspepsia is a different entity compared to FD [55, 56]. Further studies are needed to evaluate the true association between *H. pylori* infection and symptoms of FD.

19.4 Diagnostic Approach to Functional Dyspepsia

Recently, guidelines for FD were published in several countries. The committee of the American College of Gastroenterology (ACG) and the Canadian Association of Gastroenterology (CAG) issued guidance on the evaluation of FD patients [57]. Dyspeptic patients more than 60 years old should undergo prompt endoscopy to rule out esophagogastric malignancy. In patients aged 60 years or younger, the clinician may consider two approximately equivalent management options: (i) test and treat for *H. pylori* using a validated noninvasive test and, if infected, eradication therapy for *H. pylori*, or (ii) an empiric trial of acid suppression with a proton pump inhibitor (PPI) for *H. pylori*-negative or patients who remain dyspepsia after *H. pylori* eradication therapy (Fig. 19.1). However, the United European Gastroenterology (UEG) and the European Society for Neurogastroenterology and Motility (ESNM) group differs from the ACG and the CAG group in the diagnosis of FD.

According to the UEG and the ESNM consensus, endoscopy is essential for diagnosis of FD, especially in patients with alarm symptoms (Table 19.2) or risk factors [58]. However, uninvestigated dyspepsia without alarm symptoms and risk factors can be managed without endoscopy in primary care. Regarding *H. pylori* test, every patient with dyspepsia should be tested for *H. pylori* (either noninvasive or invasive test during endoscopy). Screening blood test, upper abdominal ultrasound, a gastric emptying test, or esophageal pH monitoring were not recommended for diagnosis of FD.

A recent British Society of Gastroenterology (BSG) guidelines recommended that if there is no other alarm symptoms or signs are reported, urgent endoscopy is only performed in patients aged 55 years or older with dyspepsia with weight loss, or those over 40 years of age with high-risk countries for GC or a family history of gastroesophageal cancer [59]. Nonurgent endoscopy is considered in patients aged 55 years or older with refractory dyspepsia or dyspepsia with

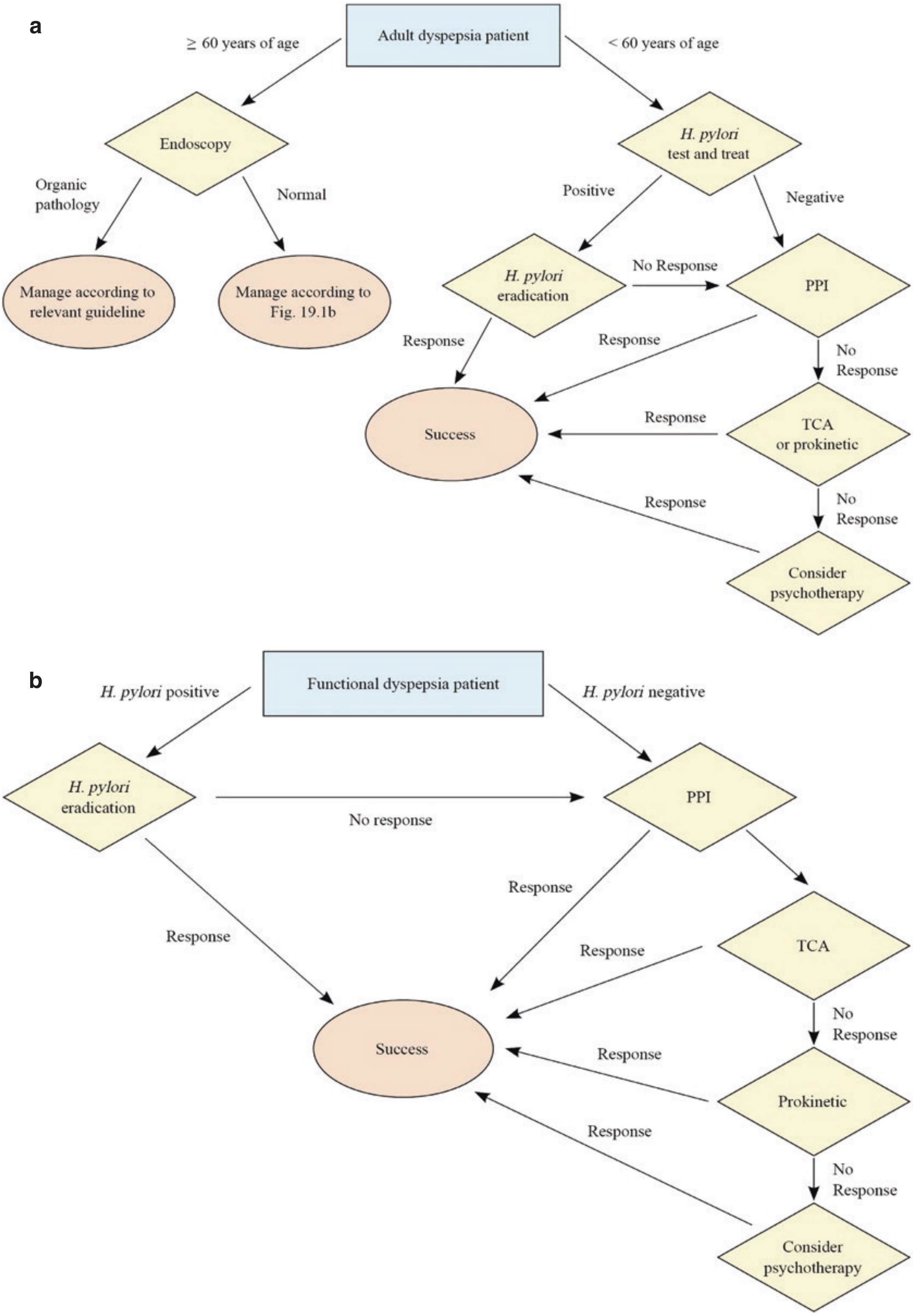
a
Adult dyspepsia patient
≥ 60 years of age
< 60 years of age
Endoscopy
Organic pathology
Normal
Manage according to relevant guideline
Manage according to Fig. 19.1b
H. pylori test and treat
Positive
Negative
H. pylori eradication
No Response
PPI
Response
Response
No Response
Success
Response
TCA or prokinetic
No Response
Response
Consider psychotherapy
b
Functional dyspepsia patient
H. pylori positive
H. pylori negative
H. pylori eradication
No response
PPI
Response
Response
TCA
Response
No Response
Response
Prokinetic
Success
Response
No Response
Consider psychotherapy

Fig. 19.1 (**a**) Management algorithm of undiagnosed dyspepsia. (**b**) Management algorithm of functional dyspepsia. *H. pylori Helicobacter pylori*, *TCA* Tricyclic antidepressants, *PPIs* Proton pump inhibitors (Adapted from Moayyedi et al. [57])

either nausea or vomiting or an elevated platelet count. Concerning *H. pylori* test, BSG group recommends noninvasive testing for *H. pylori* for every patient with dyspepsia, and, if positive, given *H. pylori* eradication therapy. In addition, a full blood test is performed in patients aged 55 years or older with dyspepsia and celiac serology in every patient with FD and overlapping IBS symptoms, and urgent abdominal CT scan is considered in patients aged 60 years or older with abdominal pain and weight loss to rule out pancreatic cancer.

In 2012, an Asian consensus report on FD was developed [61]. All of the consensus members agreed that if patients have any alarm features including new onset dyspepsia in a patient over 40 years of age in a population with high prevalence of upper gastrointestinal (UGI) malignancy, such as China, Korea, or Japan, they should undergo further testing (Fig. 19.2). Compared to

Table 19.2 Alarm features

(a)	Bleeding
(b)	Anemia
(c)	Early satiety
(e)	Progressive dysphagia
(d)	Unexplained weight loss (>10% body weight)
(f)	Odynophagia
(g)	Persistent recurrent vomiting
(h)	A family history of gastrointestinal cancer
(i)	Previous esophagogastric malignancy
(j)	Previous documented peptic ulcer
(k)	Lymphadenopathy
(l)	Abdominal mass
(m)	Fever
(n)	Jaundice
(o)	New onset dyspepsia[a]

Modified from Talley and Ford [60] and Miwa et al. [61]

[a] Miwa et al. [61] defined age of new onset dyspepsia as over 40 years of age in populations with a high prevalence of upper gastrointestinal malignancy and over 45 and 50 years in populations with intermediate and low prevalence, respectively

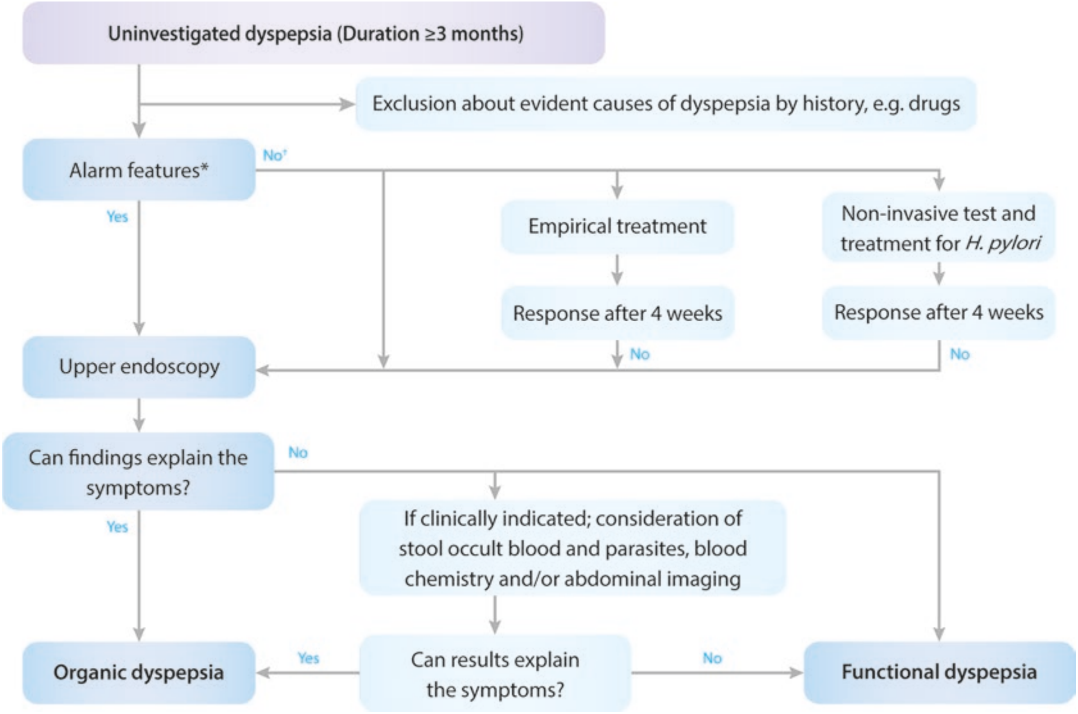
Uninvestigated dyspepsia (Duration ≥3 months)
Exclusion about evident causes of dyspepsia by history, e.g. drugs
Alarm features*
No†
Yes
Empirical treatment
Non-invasive test and treatment for H. pylori
Response after 4 weeks
Response after 4 weeks
No
No
Upper endoscopy
Can findings explain the symptoms?
No
Yes
If clinically indicated; consideration of stool occult blood and parasites, blood chemistry and/or abdominal imaging
Organic dyspepsia
Yes
Can results explain the symptoms?
No
Functional dyspepsia

Fig. 19.2 Diagnostic algorithm of functional dyspepsia. *Alarm features can be found in Table 19.2. †The appropriate choice from the three options according to patient symptoms, patient wishes, risk of *H. pylori* infection, and gastric cancer in each country as well as primary health-care settings. *H. pylori Helicobacter pylori* (Adapted from Miwa et al. [61])

the guidelines of the West, those in Asia recommend a younger patient age at which new onset of dyspepsia should prompt endoscopic examination.

However, the JSGE guidelines suggested a slightly different opinion. The JSGE guidelines stated that endoscopy is not mandatory for diagnosis of FD, because FD should be diagnosed based on a comprehensive evaluation of symptoms, age, medical history, presence of *H. pylori* infection, and laboratory findings [56]. Nonetheless, patients with positive alarm signs and suspected organic diseases should undergo endoscopy or other investigations.

According to Korean guidelines for the treatment of FD in 2020, which compared endoscopy with "test and treat" for *H. pylori*, endoscopy may be a more effective initial strategy for managing patients with FD in Korea given the high incidence of GC and low cost of endoscopy [62]. Therefore, prompt endoscopy was recommended in dyspeptic patients over 40 years of age to rule out organic diseases, such as UGI malignancy. The *H. pylori* test was recommended in dyspeptic patients who are not respond to acid suppressants or prokinetics. The usefulness of *H. pylori* serology testing before endoscopy in patients with dyspepsia was investigated in Korea [63]. The sensitivity and negative predictive value of anti-*H. pylori* IgG for organic disease were 76.6% and 85.5%, respectively, in patients with dyspepsia under 40 years old. In patients with dyspepsia over 40 years old, the sensitivity and negative predictive value of anti-*H. pylori* IgG for organic disease were 61.9% and 64.0%, respectively. Although further study will be needed, the "test-and-treat" *H. pylori* approach might not the preferred initial step in treating patients with FD in Korea. Rather, as mentioned earlier, endoscopy may be the more effective initial strategy for managing patients with FD in Korea [62, 64].

Other diagnostic tests, such as serum testing for anemia, liver disease, and pancreatitis (amylase and lipase) and upper abdominal ultrasound or CT, can be helpful in diagnosing FD [61].

19.5 Treatment of Functional Dyspepsia

There are several options for treating FD, including PPIs, histamine-2 receptor antagonists, prokinetic agents, and antidepressant and anxiolytic agents. Among them, we focus on *H. pylori* eradication.

Several epidemiologic studies have revealed that the *H. pylori* infection rate in patients with FD is higher than in matched control populations. A meta-analysis expressed a summary OR of 1.6 (95% CI, 1.4–1.8) for *H. pylori* infection in FD [65]. Although this has not yet been confirmed, this result suggests that eradication of *H. pylori* could improve FD symptoms [66].

A Cochrane meta-analysis was performed on 17 randomized controlled trials and identified an association between *H. pylori* eradication and improvement in FD symptoms. A small but significant benefit of *H. pylori* eradication therapy was observed with a number needed to treat (NNT) of 14 (95% CI, 10–25) [67]. In another study, the NNT was eight [68]. The cumulative long-term benefit of *H. pylori* eradication in patients with FD was also performed in UK. Dyspeptic symptoms in the *H. pylori* eradication group were significantly decreased compared to the placebo group, and OR was 0.84 (95% CI, 0.71–1.00) [69]. The effect of *H. pylori* eradication on Asian FD patients might be different from FD patients in the West due to the varying prevalence of *H. pylori* strains including polymorphisms of *cagA* gene, gastric acid levels, and the severity of gastritis [70]. A systemic review and meta-analysis from China reported that the summary OR for improvement in FD patients after *H. pylori* eradication was 3.61 (95% CI, 2.62–4.98) [71]. A study in Singapore demonstrated a 13-fold increased chance of FD symptom resolution in successfully eradicated *H. pylori* FD patients compared to those with persistent *H. pylori* infection (95% CI, 1.1–17.7) [72]. In addition, a meta-analysis of randomized controlled trials with 12 months follow-up revealed that dyspeptic symptoms were significantly improved in eradication group (OR 1.38; 95% CI, 1.18–1.62) [73]. Following the Cochrane meta-analysis, an updated systematic review and meta-analysis study was recently published. A total of 29 randomized controlled trials involving 6781 *H. pylori*-positive FD patients were enrolled, and follow-up was more than 3 months [74]. Eradication therapy was superior for symptom cure (relative risk [RR] of symptoms not being cured 0.91; 95% CI, 0.88–0.94, NNT = 14; 95% CI, 11–21) and symptom improvement (RR of symptoms not improving 0.84; 95% CI, 0.78–0.91; NNT = 9; 95% CI, 7–17) compared with control. In terms of success of failure o*f H. pylori* eradication therapy, successful *H. pylori* eradication therapy versus control therapy had an RR of 0.74 (95% CI, 0.64–0.85; $I^2 = 82\%$; $p < 0.001$) for noncured or nonimproving symptoms, and NNT was 6 (95% CI, 4–10). Successful versus unsuccessful eradication of *H. pylori* had an RR of 0.65 (95% CI, 0.52–0.82; $I^2 = 79\%$; $p < 0.001$) for noncured or nonimproving symptoms, and NNT was 4.5 (95% CI, 3–9). Unfortunately, there are no randomized controlled studies evaluating the effect of *H. pylori* eradication on FD in Korea. Our study group showed that *H. pylori* eradication (OR 5.81; 95% CI, 1.07–31.59) was related to improvement of FD at 1 year [75]. Studies in meta-analysis evaluated the effect of *H. pylori* eradication in patients with functional dyspepsia since 2000 and were summarized in Table 19.3.

However, Zullo et al. [79] pointed out several problems related to meta-analyses studying the effect of *H. pylori* eradication in FD patients. The type and number of dyspeptic symptoms of patients in enrolled studies were different, and the definitions of symptom improvement or regression varied widely. Many studies were performed to verify the effectiveness of *H. pylori* eradication in FD patients; however, until the early 2010s, only 12 studies were well-designed, double-blind, placebo-controlled studies with at least 50 patients followed for more than 6–12 months with final confirmation of *H. pylori* status by endoscopy with biopsy or a ^{13}C-urea breath test. Unfortunately, the type and number of symptoms and the definition of symptom improvement were different even in these 12 well-designed studies. In a recent meta-analysis, Ford et al. [74] attempted to overcome these limitations; however, the results were not significantly different. Considering these findings, we could assume that the studies evaluating the effectiveness of *H. pylori* eradication in patients with FD are difficult to interpret.

H. pylori eradication is useful in only some patients with FD. The Korean College of *Helicobacter* and Upper Gastrointestinal Research reported that *H. pylori* eradication helps long-term dyspeptic symptom improvement in some patients with FD [80]. Sugano [19] who proposed that *H. pylori*-associated FD should be separated from FD also suggested that FD patients with *H. pylori* infection should be treated before meeting criteria for a diagnosis of FD. Because *H. pylori* infection clearly influences pathological and physiological changes, residual dyspepsia symptoms after complete eradication of *H. pylori* infection might be regarded as real FD coexisting with *H. pylori* infection. Based on these considerations, the Kyoto global consensus agreed that patients who remain symptomatic after successful *H. pylori* eradication should be regarded as having FD [55] (Fig. 19.3). In addition, *H. pylori* eradication is first-line treatment for dyspeptic patients with *H. pylori* infection, because eradication therapy for dyspeptic symptoms is better than placebo in *H. pylori*-infected dyspeptic patients.

In review of recent guidelines, the concept of *H. pylori*-associated dyspepsia in the management of dyspeptic patients appears to be increasingly accepted by gastroenterologists. The ACG and the CAG recommend *H. pylori* eradication as the initial management strategy for dyspepsia in patients younger than 60 years [57]. The UEG and the ESNM groups also strongly recommend *H. pylori*-positive FD patients receive *H. pylori* eradication therapy [58]. The BSG guidelines recommend that *H. pylori* eradication therapy is one of the first-line treatments of FD, and that eradication therapy of *H. pylori* is an effective treatment for *H. pylori*-infected FD patients [59]. The JSGE guidelines also declared that dyspepsia accompanied by *H. pylori* infection should be treated as *H. pylori*-associated dyspepsia [56].

Table 19.3 Meta-analysis studies about *Helicobacter pylori* eradication in patients with functional dyspepsia since 2000

Authors	Year	Enrolled studies	Follow-up	Number	Symptom improvement	OR (95% CI)	NNT
Gisbert et al. [76]	2002	9 RCT	At least 6 months	Patients: 953 Control: 958	Eradication group: 43% (95% CI, 40–46%) Control group: 39% (95% CI, 36–42%)	1.20 (0.91–1.58)	25
Moayyedi et al. [67]	2006	17 RCT	At least 3 months	Patients: 1934 Control: 1632	Eradication group: 36% (range 15–75%) Control group: 29% (range 7–51%)	0.90 (0.86–0.94), 10% relative risk reduction (95% CI, 6–15%) in eradication group	14
Jin et al. [71]	2007	7 RCT	At least 1 month	Patients: 395 Control: 366	Eradication group: 74.4% (294/395) Control group: 47.3% (173/366)	3.61 (2.62–4.98)	NM
Zhao et al. [73]	2014	14 RCT	At least 12 months	Patients: 1490 Control: 1503	Eradication group: 40.6% (605/1490) Control group: 34.0% (511/1503)	1.38 (1.18–1.62)	15
Du et al. [77]	2016	25 RCT	At least 3 months	Patients: 2939 Control: 2468	Eradication group: 40.0% (1183/2939) Control group: 32.0% (795/2468)	1.23 (1.12–1.36)	NM
Kang et al. [78]	2019	18 RCT	At least 6 months	Patients: 2622 Control: 2152	Eradication group: 40.8% (1069/2622) Control group: 33.4% (718/2152)	1.18 (1.07–1.30)	15
Ford et al. [74]	2022	29 RCT	At least 3 months	Patients: 3625 Control: 3156	(Symptoms not being cured or improving in) Eradication group: 64.8% (2350/3625) Control group: 76.1% (2402/3156)	Relative risk of symptoms not being cured or improving: 0.87 (0.83–0.92)	10

OR Odds ratio, *CI* Confidence interval, *NNT* Number needed to treat, *RCT* Randomized controlled trials, *NM* Not mentioned

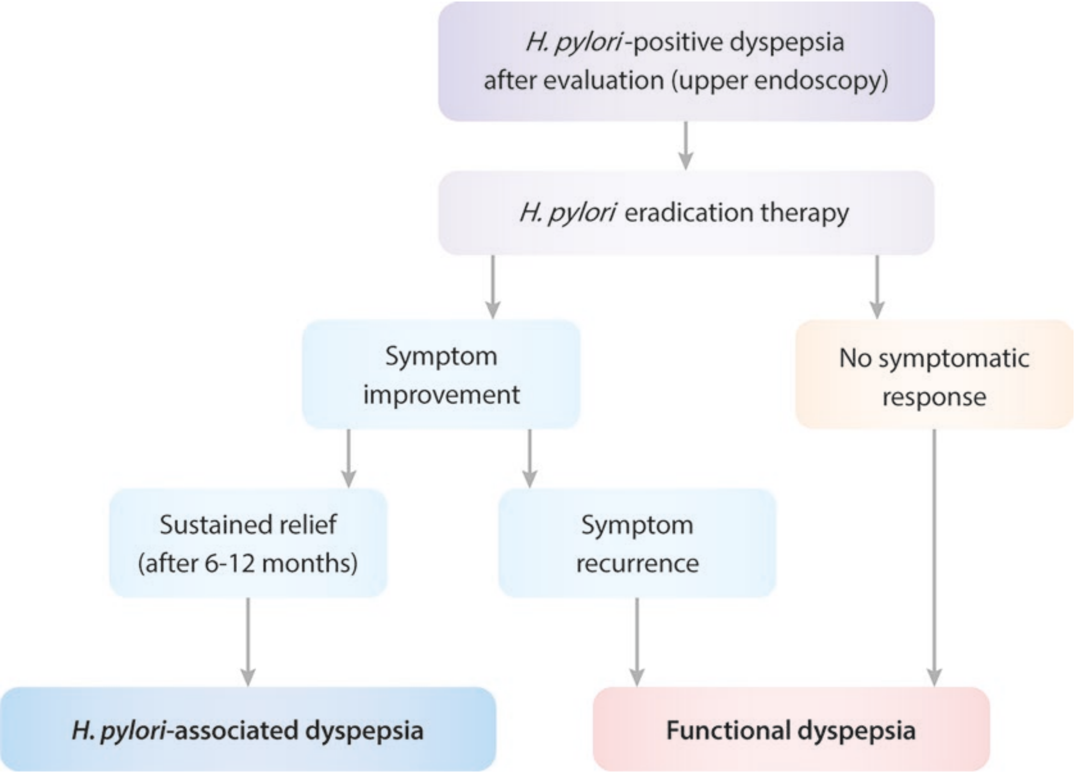
H. pylori-positive dyspepsia after evaluation (upper endoscopy)
H. pylori eradication therapy
Symptom improvement
No symptomatic response
Sustained relief (after 6-12 months)
Symptom recurrence
H. pylori-associated dyspepsia
Functional dyspepsia

Fig. 19.3 Diagnostic algorithm of *H. pylori*-associated dyspepsia. *H. pylori Helicobacter pylori* (Adapted from Sugano et al. [55])

All things considered, *H. pylori* eradication is effective in some patients with dyspepsia. Considering the short duration and acceptable cost-benefit analysis for improving dyspeptic symptoms, *H. pylori* eradication could be proposed as the treatment for those with *H. pylori* infection.

19.6 Conclusions

FD is one of the most prevalent GI disorders; however, many physicians are hard to diagnose FD due to the varied pathophysiology and symptoms. Among the many causes of FD, *H. pylori* infection is considered to have some effect on the onset of FD worldwide. *H. pylori* eradication is suggested as one of the therapeutic options for FD patients with *H. pylori* infection, and several meta-analyses reported that *H. pylori* eradication improved dyspeptic symptoms in FD patients compared to placebo. Thus, *H. pylori* eradication could be attempted for treatment of FD patients. Some limitations still exist, such as lack of well-designed, double-blinded, placebo-controlled studies to establish the effectiveness of *H. pylori* eradication in FD patients. Therefore, additional well-designed, prospective studies are needed to demonstrate the association between FD and *H. pylori* infection.

References

1. Ford AC, Marwaha A, Sood R, Moayyedi P. Global prevalence of, and risk factors for, uninvestigated dyspepsia: a meta-analysis. Gut. 2015;64:1049–57.
2. Kim JS, Lee KJ, Kim JH, Hahm KB, Cho SW. Functional gastrointestinal disorders in patients referred to specialist gastroenterologists in a tertiary hospital. Korean J Neurogastroenterol Motil. 2004;10:111–7.

3. Jung HK, Jang B, Kim YH, Park J, Park SY, Nam MH, et al. Health care costs of digestive diseases in Korea. Korean J Gastroenterol. 2011;58:323–31.
4. Aziz I, Palsson OS, Tornblom H, Sperber AD, Whitehead WE, Simren M. Epidemiology, clinical characteristics, and associations for symptom-based Rome IV functional dyspepsia in adults in the USA, Canada, and the UK: a cross-sectional population-based study. Lancet Gastroenterol Hepatol. 2018;3:252–62.
5. Stanghellini V, Chan FK, Hasler WL, Malagelada JR, Suzuki H, Tack J, et al. Gastroduodenal disorders. Gastroenterology. 2016;150:1380–92.
6. Choung RS, Locke GR, Schleck CD, Zinsmeister AR, Talley NJ. Do distinct dyspepsia subgroups exist in the community? A population-based study. Am J Gastroenterol. 2007;102:1983–9.
7. Aro P, Talley NJ, Ronkainen J, Storskrubb T, Vieth M, Johansson SE, et al. Anxiety is associated with uninvestigated and functional dyspepsia (Rome III criteria) in a Swedish population-based study. Gastroenterology. 2009;137:94–100.
8. Zagari RM, Law GR, Fuccio L, Cennamo V, Gilthorpe MS, Forman D, et al. Epidemiology of functional dyspepsia and subgroups in the Italian general population: an endoscopic study. Gastroenterology. 2010;138:1302–11.
9. Dizdar V, Gilja OH, Hausken T. Increased visceral sensitivity in Giardia-induced postinfectious irritable bowel syndrome and functional dyspepsia. Effect of the 5HT3-antagonist ondansetron. Neurogastroenterol Motil. 2007;19:977–82.
10. Hanevik K, Hausken T, Morken MH, Strand EA, Morch K, Coll P, et al. Persisting symptoms and duodenal inflammation related to Giardia duodenalis infection. J Infect. 2007;55:524–30.
11. Futagami S, Itoh T, Sakamoto C. Systematic review with meta-analysis: post-infectious functional dyspepsia. Aliment Pharmacol Ther. 2015;41:177–88.
12. Pike BL, Porter CK, Sorrell TJ, Riddle MS. Acute gastroenteritis and the risk of functional dyspepsia: a systematic review and meta-analysis. Am J Gastroenterol. 2013;108:1558–63.
13. Gwee KA. Post-infectious irritable bowel syndrome, an inflammation-immunological model with relevance for other IBS and functional dyspepsia. J Neurogastroenterol Motil. 2010;16:30–4.
14. Suzuki H, Hibi T, Marshall BJ. *Helicobacter pylori*: present status and future prospects in Japan. J Gastroenterol. 2007;42:1–15.
15. D'Elios MM, Andersen LP. Inflammation, immunity, and vaccines for *Helicobacter pylori*. Helicobacter. 2009;14 (Suppl 1):21–8.
16. Suzuki H, Matsuzaki J, Hibi T. What is the difference between *Helicobacter pylori*-associated dyspepsia and functional dyspepsia? J Neurogastroenterol Motil. 2011;17:124–30.
17. Liu Y, Vosmaer GD, Tytgat GN, Xiao SD, Ten Kate FJ. Gastrin (G) cells and somatostatin (D) cells in patients with dyspeptic symptoms: *Helicobacter pylori* associated and non-associated gastritis. J Clin Pathol. 2005;58:927–31.
18. Konturek SJ, Brzozowski T, Konturek PC, Schubert ML, Pawlik WW, Padol S, et al. Brain-gut and appetite regulating hormones in the control of gastric secretion and mucosal protection. J Physiol Pharmacol. 2008;59 (Suppl 2):7–31.
19. Sugano K. Should we still subcategorize *Helicobacter pylori*-associated dyspepsia as functional disease? J Neurogastroenterol Motil. 2011;17:366–71.
20. Azuma T. *Helicobacter pylori* CagA protein variation associated with gastric cancer in Asia. J Gastroenterol. 2004;39:97–103.
21. Yamaoka Y, Kato M, Asaka M. Geographic differences in gastric cancer incidence can be explained by differences between *Helicobacter pylori* strains. Intern Med. 2008;47:1077–83.
22. Czinn SJ, Bertram TA, Murray PD, Yang P. Relationship between gastric inflammatory response and symptoms in patients infected with *Helicobacter pylori*. Scand J Gastroenterol Suppl. 1991;181:33–7.
23. van der Schaar PJ, Straathof JW, Veenendaal RA, Lamers CB, Masclee AA. Does *Helicobacter pylori* gastritis affect motor function of proximal stomach in dyspeptic patients? Dig Dis Sci. 2001;46:1833–8.
24. Pereira-Lima JG, Scholl J, Pinheiro JB, Pereira-Lima L, Riemann JF. *Helicobacter pylori*-associated gastritis: does it play a role in functional dyspepsia? Z Gastroenterol. 1995;33:421–5.
25. Joshi A, Gupta SD, Ahuja V, Sharma MP. Symptom score does not correlate with gastritis grade and *Helicobacter pylori* infection in non ulcer dyspepsia. Trop Gastroenterol. 2001;22:194–6.
26. Wren AM, Bloom SR. Gut hormones and appetite control. Gastroenterology. 2007;132:2116–30.
27. Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, et al. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. Gastroenterology. 2002;123:1120–8.
28. Fukuhara S, Suzuki H, Masaoka T, Arakawa M, Hosoda H, Minegishi Y, et al. Enhanced ghrelin secretion in rats with cysteamine-induced duodenal ulcers. Am J Physiol Gastrointest Liver Physiol. 2005;289:G138–45.
29. Suzuki H, Masaoka T, Hosoda H, Nomura S, Ohara T, Kangawa K, et al. Plasma ghrelin concentration correlates with the levels of serum pepsinogen I and pepsinogen I/II ratio—a possible novel and non-invasive marker for gastric atrophy. Hepato-Gastroenterology. 2004;51:1249–54.
30. Kawashima J, Ohno S, Sakurada T, Takabayashi H, Kudo M, Ro S, et al. Circulating acylated ghrelin level decreases in accordance with the extent of atrophic gastritis. J Gastroenterol. 2009;44:1046–54.
31. Mantero P, Matus GS, Corti RE, Cabanne AM, Zerbetto de Palma GG, Marchesi Olid L, et al. *Helicobacter pylori* and corpus gastric pathology are associated with lower serum ghrelin. World J Gastroenterol. 2018;24:397–407.

32. Takamori K, Mizuta Y, Takeshima F, Akazawa Y, Isomoto H, Ohnita K, et al. Relation among plasma ghrelin level, gastric emptying, and psychologic condition in patients with functional dyspepsia. J Clin Gastroenterol. 2007;41:477–83.
33. Shinomiya T, Fukunaga M, Akamizu T, Irako T, Yokode M, Kangawa K, et al. Plasma acylated ghrelin levels correlate with subjective symptoms of functional dyspepsia in female patients. Scand J Gastroenterol. 2005;40:648–53.
34. Lee KJ, Cha DY, Cheon SJ, Yeo M, Cho SW. Plasma ghrelin levels and their relationship with gastric emptying in patients with dysmotility-like functional dyspepsia. Digestion. 2009;80:58–63.
35. Shindo T, Futagami S, Hiratsuka T, Horie A, Hamamoto T, Ueki N, et al. Comparison of gastric emptying and plasma ghrelin levels in patients with functional dyspepsia and non-erosive reflux disease. Digestion. 2009;79:65–72.
36. Yamawaki H, Futagami S, Shimpuku M, Shindo T, Maruki Y, Nagoya H, et al. Leu72Met408 polymorphism of the ghrelin gene is associated with early phase of gastric emptying in the patients with functional dyspepsia in Japan. J Neurogastroenterol Motil. 2015;21:93–102.
37. Akamizu T, Iwakura H, Ariyasu H, Hosoda H, Murayama T, Yokode M, et al. Repeated administration of ghrelin to patients with functional dyspepsia: its effects on food intake and appetite. Eur J Endocrinol. 2008;158:491–8.
38. Lanzini A, Magni P, Petroni ML, Motta M, Lanzarotto F, Villanacci V, et al. Circulating ghrelin level is increased in coeliac disease as in functional dyspepsia and reverts to normal during gluten-free diet. Aliment Pharmacol Ther. 2006;23:907–13.
39. Choi YJ, Kim N, Yoon H, Shin CM, Park YS, Park JH, et al. Increase in plasma acyl ghrelin levels is associated with abatement of dyspepsia following *Helicobacter pylori* eradication. J Gastroenterol. 2016;51:548–59.
40. Sanger GJ, Lee K. Hormones of the gut-brain axis as targets for the treatment of upper gastrointestinal disorders. Nat Rev Drug Discov. 2008;7:241–54.
41. Lankarani KB, Moghadami M, Masoumpoor M, Geramizadeh B, Omrani GR. Serum leptin level in patients with functional dyspepsia. Dig Liver Dis. 2004;36:717–21.
42. Azuma T, Suto H, Ito Y, Ohtani M, Dojo M, Kuriyama M, et al. Gastric leptin and *Helicobacter pylori* infection. Gut. 2001;49:324–9.
43. Chiloiro M, Russo F, Riezzo G, Leoci C, Clemente C, Messa C, et al. Effect of *Helicobacter pylori* infection on gastric emptying and gastrointestinal hormones in dyspeptic and healthy subjects. Dig Dis Sci. 2001;46:46–53.
44. Van den Houte K, Scarpellini E, Verbeure W, Mori H, Schol J, Masuy I, et al. The role of GI peptides in functional dyspepsia and gastroparesis: a systematic review. Front Psych. 2020;11:172.
45. Talley NJ, Walker MM, Aro P, Ronkainen J, Storskrubb T, Hindley LA, et al. Non-ulcer dyspepsia and duodenal eosinophilia: an adult endoscopic population-based case-control study. Clin Gastroenterol Hepatol. 2007;5:1175–83.
46. Aydemir SA, Tekin IO, Numanoglu G, Borazan A, Ustundag Y. Eosinophil infiltration, gastric juice and serum eosinophil cationic protein levels in *Helicobacter pylori*-associated chronic gastritis and gastric ulcer. Mediat Inflamm. 2004;13:369–72.
47. Walker MM, Aggarwal KR, Shim LS, Bassan M, Kalantar JS, Weltman MD, et al. Duodenal eosinophilia and early satiety in functional dyspepsia: confirmation of a positive association in an Australian cohort. J Gastroenterol Hepatol. 2014;29:474–9.
48. Shah A, Fairlie T, Brown G, Jones MP, Eslick GD, Duncanson K, et al. Duodenal eosinophils and mast cells in functional dyspepsia: a systematic review and meta-analysis of case-control studies. Clin Gastroenterol Hepatol. 2022;20:2229–42.e29.
49. Gargala G, Lecleire S, Francois A, Jacquot S, Dechelotte P, Ballet JJ, et al. Duodenal intraepithelial T lymphocytes in patients with functional dyspepsia. World J Gastroenterol. 2007;13:2333–8.
50. Maldonado-Contreras A, Goldfarb KC, Godoy-Vitorino F, Karaoz U, Contreras M, Blaser MJ, et al. Structure of the human gastric bacterial community in relation to *Helicobacter pylori* status. ISME J. 2011;5:574–9.
51. Gantuya B, El-Serag HB, Matsumoto T, Ajami NJ, Oyuntsetseg K, Azzaya D, et al. Gastric microbiota in *Helicobacter pylori*-negative and -positive gastritis among high incidence of gastric cancer area. Cancers (Basel). 2019;11:504.
52. Tziatzios G, Gkolfakis P, Papanikolaou IS, Mathur R, Pimentel M, Giamarellos-Bourboulis EJ, et al. Gut microbiota dysbiosis in functional dyspepsia. Microorganisms. 2020;8:691.
53. Zhong L, Shanahan ER, Raj A, Koloski NA, Fletcher L, Morrison M, et al. Dyspepsia and the microbiome: time to focus on the small intestine. Gut. 2017;66:1168–9.
54. Shanahan ER, Kang S, Staudacher H, Shah A, Do A, Burns G, et al. Alterations to the duodenal microbiota are linked to gastric emptying and symptoms in functional dyspepsia. Gut. 2023;72:929–38.
55. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, et al. Kyoto global consensus report on *Helicobacter pylori* gastritis. Gut. 2015;64:1353–67.
56. Miwa H, Nagahara A, Asakawa A, Arai M, Oshima T, Kasugai K, et al. Evidence-based clinical practice guidelines for functional dyspepsia 2021. J Gastroenterol. 2022;57:47–61.
57. Moayyedi P, Lacy BE, Andrews CN, Enns RA, Howden CW, Vakil N. ACG and CAG clinical guideline: management of dyspepsia. Am J Gastroenterol. 2017;112:988–1013.
58. Wauters L, Dickman R, Drug V, Mulak A, Serra J, Enck P, et al. United European gastroenterology (UEG) and European Society for Neurogastroenterology and

Motility (ESNM) consensus on functional dyspepsia. United European Gastroenterol J. 2021;9:307–31.

59. Black CJ, Paine PA, Agrawal A, Aziz I, Eugenicos MP, Houghton LA, et al. British Society of Gastroenterology guidelines on the management of functional dyspepsia. Gut. 2022;71:1697–723.
60. Talley NJ, Ford AC. Functional dyspepsia. N Engl J Med. 2015;373:1853–63.
61. Miwa H, Ghoshal UC, Gonlachanvit S, Gwee KA, Ang TL, Chang FY, et al. Asian consensus report on functional dyspepsia. J Neurogastroenterol Motil. 2012;18:150–68.
62. Oh JH, Kwon JG, Jung HK, Tae CH, Song KH, Kang SJ, et al. Clinical practice guidelines for functional dyspepsia in Korea. J Neurogastroenterol Motil. 2020;26:29–50.
63. Hwang IR, Kim JH, Lee KJ, Cho SW. Can *Helicobacter pylori* serology predict non-ulcer dyspepsia in young dyspeptic patients? Korean J Gastrointest Endosc. 2000;21:696–703.
64. Park MI. Diagnosis & management of dyspepsia. Korean J Med. 2009;76 (Suppl 2):S298–303.
65. Jaakkimainen RL, Boyle E, Tudiver F. Is *Helicobacter pylori* associated with non-ulcer dyspepsia and will eradication improve symptoms? A meta-analysis. BMJ. 1999;319:1040–4.
66. Miwa H, Hirai S, Nagahara A, Murai T, Nishira T, Kikuchi S, et al. Cure of *Helicobacter pylori* infection does not improve symptoms in non-ulcer dyspepsia patients-a double-blind placebo-controlled study. Aliment Pharmacol Ther. 2000;14:317–24.
67. Moayyedi P, Soo S, Deeks J, Delaney B, Harris A, Innes M, et al. Eradication of *Helicobacter pylori* for non-ulcer dyspepsia. Cochrane Database Syst Rev. 2006:CD002096.
68. Mazzoleni LE, Sander GB, Francesconi CF, Mazzoleni F, Uchoa DM, De Bona LR, et al. *Helicobacter pylori* eradication in functional dyspepsia: HEROES trial. Arch Intern Med. 2011;171:1929–36.
69. Harvey RF, Lane JA, Nair P, Egger M, Harvey I, Donovan J, et al. Clinical trial: prolonged beneficial effect of *Helicobacter pylori* eradication on dyspepsia consultations—the Bristol Helicobacter Project. Aliment Pharmacol Ther. 2010;32:394–400.
70. Suzuki H, Masaoka T, Sakai G, Ishii H, Hibi T. Improvement of gastrointestinal quality of life scores in cases of *Helicobacter pylori*-positive functional dyspepsia after successful eradication therapy. J Gastroenterol Hepatol. 2005;20:1652–60.
71. Jin X, Li YM. Systematic review and meta-analysis from Chinese literature: the association between *Helicobacter pylori* eradication and improvement of functional dyspepsia. Helicobacter. 2007;12:541–6.
72. Gwee KA, Teng L, Wong RK, Ho KY, Sutedja DS, Yeoh KG. The response of Asian patients with functional dyspepsia to eradication of *Helicobacter pylori* infection. Eur J Gastroenterol Hepatol. 2009;21:417–24.
73. Zhao B, Zhao J, Cheng WF, Shi WJ, Liu W, Pan XL, et al. Efficacy of *Helicobacter pylori* eradication therapy on functional dyspepsia: a meta-analysis of randomized controlled studies with 12-month follow-up. J Clin Gastroenterol. 2014;48:241–7.
74. Ford AC, Tsipotis E, Yuan Y, Leontiadis GI, Moayyedi P. Efficacy of *Helicobacter pylori* eradication therapy for functional dyspepsia: updated systematic review and meta-analysis. Gut. 2022;71:1967–75.
75. Kim SE, Park YS, Kim N, Kim MS, Jo HJ, Shin CM, et al. Effect of *Helicobacter pylori* eradication on functional dyspepsia. J Neurogastroenterol Motil. 2013;19:233–43.
76. Gisbert JP, Calvet X, Gabriel R, Pajares JM. *Helicobacter pylori* infection and functional dyspepsia. Meta-analysis of efficacy of eradication therapy. Med Clin (Barc). 2002;118:405–9.
77. Du LJ, Chen BR, Kim JJ, Kim S, Shen JH, Dai N. *Helicobacter pylori* eradication therapy for functional dyspepsia: systematic review and meta-analysis. World J Gastroenterol. 2016;22:3486–95.
78. Kang SJ, Park B, Shin CM. *Helicobacter pylori* eradication therapy for functional dyspepsia: a meta-analysis by region and *H. pylori* prevalence. J Clin Med. 2019;8:1324.
79. Zullo A, Hassan C, De Francesco V, Repici A, Manta R, Tomao S, et al. *Helicobacter pylori* and functional dyspepsia: an unsolved issue? World J Gastroenterol. 2014;20:8957–63.
80. Jung HK, Kang SJ, Lee YC, Yang HJ, Park SY, Shin CM, et al. Evidence-based guidelines for the treatment of *Helicobacter pylori* infection in Korea 2020. Gut Liver. 2021;15:168–95.

20

Peptic Ulcer

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Abstract

The incidence and prevalence of peptic ulcer have been decreasing with the advancement of medical management and low prevalence of *Helicobacter pylori* (*H. pylori*) infection. However, complications of peptic ulcer are still common, and admission rate has not been decreasing with the wide use of nonsteroidal anti-inflammatory drugs (NSAIDs) and antiplatelet agents. *H. pylori* and NSAIDs are the most common and important etiologies of peptic ulcer. Early diagnosis with endoscopic and radiologic examinations is important for the prevention of complications. Similar endoscopic features with peptic ulcer can be seen with malignancy and should be excluded for the differential diagnosis. Elimination of causative factors and proton pump inhibitor (PPI)-based medications are main treatment strategies. Potassium competitive acid blocker, which inhibits gastric H^+-K^+-ATPase is expected to partially supplement the unmet clinical needs of PPI and showed a noninferior efficacy compared to PPI for the treatment of gastric ulcers and the prevention of peptic ulcers in NSAID users. High-risk factors of NSAID-induced peptic ulcer and its complications include old age, a history of peptic ulcer, administration of high-dose NSAIDs, and coadministration of aspirin, antiplatelet agent, anticoagulants or steroid. Patients with long-term NSAID or low-dose aspirin therapy are recommended testing for, and treatment of *H. pylori* infection to prevent peptic ulcer and its complications. These patients with high-risk factors are also recommended to receive low-dose PPIs to prevent peptic ulcer and its complications. The risk of cardiovascular diseases should be considered in high-risk patients who are taking NSAIDs, and these patients with low-risk of cardiovascular diseases are recommended to administrate cyclooxygenase-2 inhibitor as NSAID. The clinical characteristics of idiopathic or non-*H. pylori*, non-NSAID ulcer need more investigation.

Keywords

Helicobacter pylori · Nonsteroidal anti-inflammatory agents · Peptic ulcer

20.1 Introduction

Gastrointestinal ulcers can be found anywhere in the gastrointestinal tract from the esophagus to colon, and various causative factors are related to

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the development of ulceration. Peptic ulcer usually refers to mucosal injury more than 5 mm diameter confined to the mucosa and submucosa in the stomach and duodenum [1]. Since digestive enzyme pepsin involved in gastric acid secretion had been suspected as the main responsible factor for the development of ulcers, terminology of peptic ulcer has been used. This chapter will review about epidemiology, etiology, clinical features, diagnosis, treatments, and complications of peptic ulcer.

20.2 Epidemiology

The incidence and prevalence of peptic ulcer have been decreasing [2]. Hygiene improvement and low prevalence of *Helicobacter pylori* (*H. pylori*) infection are suspected as main reasons. The annual global incidence is reported as 0.1–1.5%, and prevalence is reported as 0.03–0.19% [2, 3]. However, there are also reports indicating that complications of peptic ulcer are still common and admission rate has not been decreasing [4, 5]. The reasons are supposed to be wide use of NSAIDs or aspirin, especially in elderly patients [4–6].

According to the Korean studies, the prevalence of peptic ulcer is estimated as 2–3% [7–9], and it has not been decreasing [10, 11]. The prevalence of duodenal ulcer is reported to be constant or slightly decreasing, but that of gastric ulcer is reported to be increasing [11, 12]. The mean age of the patients showed higher in gastric ulcer than that of duodenal ulcer, and patients with gastric ulcer take more NSAIDs or aspirin [12].

20.3 Causative Factors

The pathophysiology of peptic ulcer had been suspected as an imbalance between mucosal injury factors and mucoprotective defensive factors. However, *H. pylori* infection and NSAIDs or aspirin use are known to account for more than 70% and considered as the most important causative factors of peptic ulcer now [1]. Attention to idiopathic or non-*H. pylori*, non-NSAID peptic ulcer is increasing because of high recurrence and mortality rates [13, 14].

20.3.1 *H. pylori* Infection

Although *H. pylori* infection is still the most important causative factor of peptic ulcer, the seroprevalence of *H. pylori* in Korean population (over 16 years) decreased from 66.9% in 1998 to 59.6% in 2005 [15]. The detection rate of *H. pylori* showed slightly higher in duodenal ulcer as 73–100% than that of gastric ulcer as 65–100% [16–21]. However, another study reported increased detection rate from 66.1 to 73.1% in gastric ulcer (from the 1990s to 2000s) and decreased detection rate from 79.3 to 68.1% in duodenal ulcer (from the 1990s to 2000s) [12]. Several theories have been proposed for the development of duodenal ulcer. Increased acid secretion by colonized *H. pylori* in gastric metaplasia developed in duodenal mucosa and in the gastric antrum developed as antral-dominant gastritis has been proposed. Decreased bicarbonate secretion by *H. pylori* in the duodenum has been also suspected as one of the pathogeneses. Gastric ulcer is characterized by normal or decreased acid secretion. Defect in mucosal protective function and reflux of duodenal digestive enzymes by loosening of pyloric sphincter have been suspected as the pathogenesis of gastric ulcer [1]. Only about 5–10% of patients with *H. pylori* infection have peptic ulcer. Thus, variation of bacterial characteristics itself, host immunity, and unknown factors other than *H. pylori* infection are suspected to be influential for the development of peptic ulcer [1].

20.3.2 NSAIDs

With the aging of the population, NSAIDs or aspirin is widely used in patients with chronic musculoskeletal or cardiovascular diseases.

Long-term use of NSAIDs has been reported to increase the risk of peptic ulcer and complications [22]. Low-dose aspirin, which is widely used for the prevention of cardiovascular diseases, is also known to increase the risk of peptic ulcer and complications [23].

There are various pathophysiologic mechanisms for the development of peptic ulcer in patients taking NSAIDs. The main mechanism is inhibition of cyclooxygenase (COX) (mainly COX-1) by NSAIDs, followed by decreased production of prostaglandin, which is responsible for mucosal protection [24]. Direct mucosal injury by NSAIDs and increased inflammatory response by activation of lipoxygenase (LOX) are suspected as another mechanism.

Risk factors for the development of peptic ulcer in NSAID users are past medical history of peptic ulcer or complications, higher dose, long-term use, old age, other comorbidities, and coadministration of steroid or anticoagulants [25–27]. NSAID use and *H. pylori* infection are independent risk factors for the development of peptic ulcer. Patients with both risk factors are known to have a higher risk for the development of peptic ulcer.

20.3.3 Non-*H. pylori*, Non-NSAID Ulcer

Attention to idiopathic or non-*H. pylori*, non-NSAID peptic ulcer is increasing. This is because the recurrence rate is high (35% within 2 years, 42% within 7 years) in non-*H. pylori* peptic ulcer patients and the definite cause is not identified in 28% of non-NSAID ulcer patients [12–14, 28].

The prevalence is estimated as 16.2–35.7% in Korean studies, although the geographical difference exists [12, 28, 29]. The presumptive causes are as follows: increased gastric acid secretion; smoking; malignancy; use of antiplatelet agents (clopidogrel, ticlopidine, prasugrel, etc.), potassium chloride, and bisphosphonate; cytomegalovirus infection; Herpes simplex virus infection; tuberculosis; syphilis; portal hypertension; Behcet's disease; stress; and other comorbidities [30–35]. Although suspected causes are listed above, the possibility of false-negative results for *H. pylori* infection should be considered, and unrecognized use of NSAIDs should be excluded through the meticulous history taking.

Among the causative factors of peptic ulcer development, the frequency is relatively low in this group of patients. However, more investigation is needed for these patients, because they usually need sustained antiulcer treatments.

20.4 Clinical Features

Patients with peptic ulcer show gastrointestinal symptoms, such as abdominal pain or dyspepsia. However, no symptom is known to be sensitive or specific for the diagnosis or prediction of complications of peptic ulcer. Especially, patients with silent ulcer who have decreased visceral sensitivity show no symptoms. Thus, diagnosis or making a decision of diagnostic procedure based on clinical symptoms has limitations [36].

The abdominal pain can be heartburning or colicky. It could accompany reflux symptom or dyspepsia. The symptom of duodenal ulcer develops in the acid secretion state without food buffer. Ingested food is expelled from the stomach within 2–3 h, and gastric acid secretion is sustained for 3–5 h after food intake. Thus, symptom of duodenal ulcer usually develops 2–5 h after food intake. The characteristic symptoms are midnight abdominal pain when the gastric acid secretion is active or pain relieved by food or antacid intake. In contrast to the duodenal ulcer, patients with gastric ulcer show abdominal pain lasting for 1–2 h after food intake and relieving by food passage from the stomach.

20.5 Diagnosis

Endoscopic examination is recommended for the patients with clinical symptoms, risk factors, or who suspected for the development of complications of peptic ulcer (Fig. 20.1). Gastric cancer

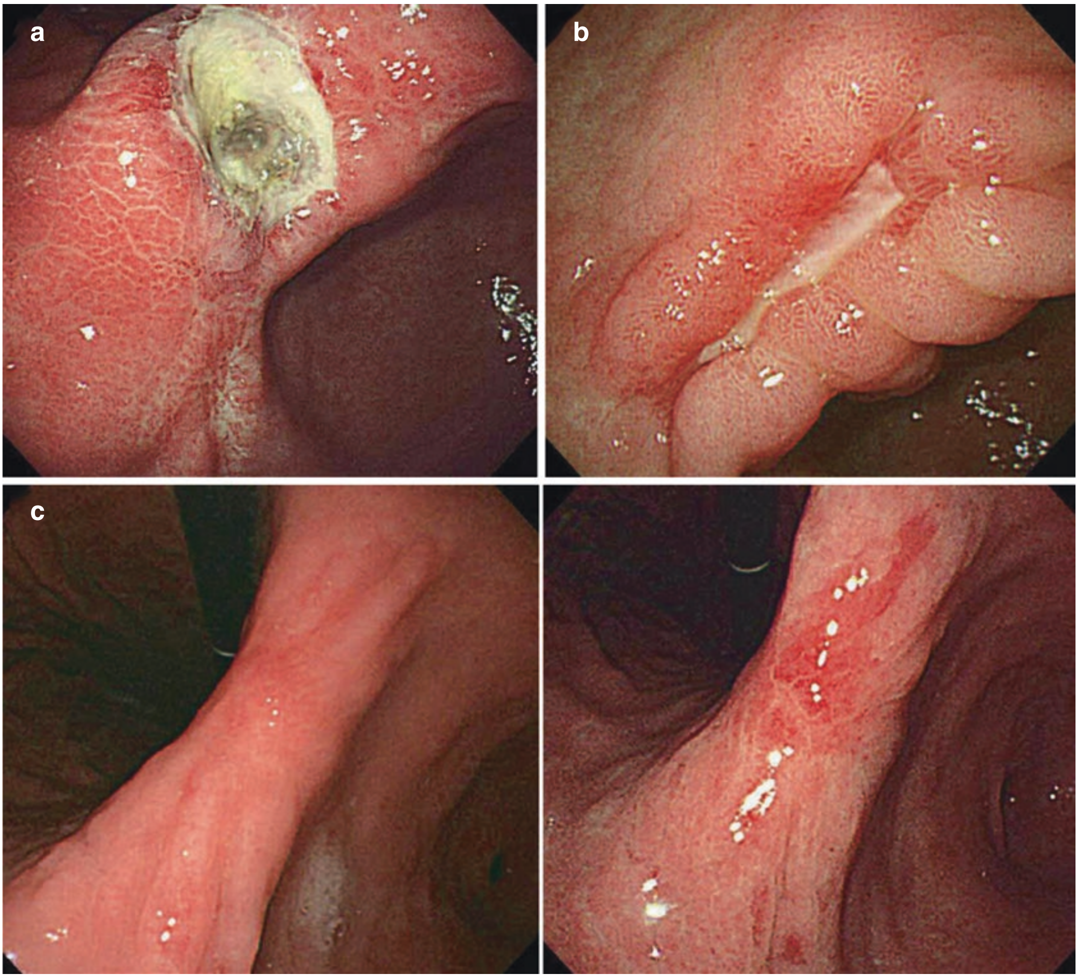

Fig. 20.1 Endoscopic feature of peptic ulcer. (**a**) Active stage gastric ulcer on angle which has regular margin and edge with hemorrhagic stigmata on the base of ulcer. (**b**) Healing stage gastric ulcer on angle with round edges and regular regenerative epithelium. (**c**) Scar stage gastric ulcer on angle (*left*, *white scar*; *right*, *red scar*)

(GC) is prevalent in East Asia including Korea. Early detection and treatment by endoscopic resection in the specific subset of early GC have shown similar treatment outcome with surgery. Gastric ulcer should be followed up by endoscopic examination, and malignancy should be excluded by histologic examination, because of similar morphologic life cycle of early GC [37] (Table 20.1). Advanced GC shows irregular appearance (relatively irregular edge and margin), but differential diagnosis is sometimes necessary in Borrmann type II or III categories [38]. In addition to the diagnosis of peptic ulcer by endoscopy, history taking for the causative factors, such as NSAID intake and tests for *H. pylori* infection, is needed.

Table 20.1 Endoscopic morphology of benign ulcer and malignancy

	Peptic ulcer	Malignancy
Folds	Regular folds that extend to the margins of the base	Prominent folds that do not extend to the margins
	Radiation of smooth, symmetric folds to the ulcer crater	Interrupted, nodular, cutting, fused, or clubbing-shaped folds
Margin	Smooth margin with round edges, regular regenerative epithelium, and normal surrounding mucosa	Overhanging, irregular, thickened, nodular, asymmetrical hyperemic, discolored or moth-eaten margin
Base	Flat and regular base	Necrotic or hemorrhagic base with some islands of regenerative epithelium

20.6 Treatment

The aim of treatment is symptomatic relief and prevention of complications and recurrence with the healing of ulceration. Medical treatments are a mainstay, but complications usually need additional endoscopic management. Proton pump inhibitor (PPI), which is the most potent acid secretion blocker of the gastric parietal cell, is used. Antacid and mucosal protective agents are also used for the treatment of peptic ulcer. A total of 4–8 weeks of PPIs are used, including an eradication period for the treatment of peptic ulcer. Six to eight weeks for gastric ulcer and 4–6 weeks for duodenal ulcer are generally recommended for the treatment period of peptic ulcer [39]. Duodenal ulcer without complications is not recommended for the sustained use of PPI. However, in cases of peptic ulcer with complications, sustained use of PPI is recommended [40].

Removal of causative factors is essential for the treatment and prevention of complications. *H. pylori* eradication is the most effective management for the healing of ulcer and prevention of recurrence in the *H. pylori*-associated peptic ulcer. In the NSAID-induced peptic ulcer, medical managements with stopping NSAIDs are recommended. In cases when discontinuing NSAIDs are impossible, combination of PPI or misoprostol with NSAIDs is recommended, and PPI showed better efficacy than misoprostol [41].

In cases of NSAID-induced peptic ulcer, the risk of peptic ulcer complications is increased when the *H. pylori* infection is present. On the contrary, there have been reports about the beneficial effect of *H. pylori* infection in patients with NSAID-induced peptic ulcer through increasing COX-2 in the gastric mucosa, which is responsible for the mucosal healing [42, 43]. Therefore, *H. pylori* eradication is recommended after healing of ulcers in patients with NSAID-induced gastric ulcer [39].

Stopping antiplatelet agent, including aspirin, prevents complications of peptic ulcer. However, it could induce or aggravate cardiovascular or cerebrovascular events. Therefore, balancing risk and benefit of continuing or stopping medication and personalized evaluation are important [27].

In the non-*H. pylori*, non-NSAID peptic ulcers, the possibility of *H. pylori* infection should be considered, and unrecognized use of NSAIDs should be evaluated. PPI is usually used for 4–8 weeks. The treatment response is relatively low in this group of patients. Therefore, sustained treatments are frequently required [44, 45].

Refractory ulcer is defined as nonresponse to 8–12 weeks of antisecretory treatments (12 weeks for gastric ulcer and 8 weeks for duodenal ulcer). Adherence to drug, smoking history, use of NSAIDs, Crohn's disease, Zollinger-Ellison syndrome, and other comorbidities should be evaluated. Follow-up endoscopic examination with histology to exclude malignancy is important.

Potassium competitive acid blocker (P-CAB), a selective inhibitor of gastric H^+-K^+-ATPase inhibits gastric acid secretion with a pharmacological mechanism different from PPI. PPIs need to undergo chemical transformation into their active form and need simultaneous activation of H^+-K^+-ATPase on the parietal cell membrane for gastric acid inhibition, attach to the target molecule irreversibly, and are eliminated from plasma rapidly [46, 47].

PPI is a weakly basic medication with a slow onset of action, which requires an enteric coating formulation to be absorbed in the small intestine when administered orally (3–5 days required to reach maximum effect). CYP2C19 genetic polymorphism might also influence the effect of PPI during metabolism [46, 47].

P-CAB, on the other hand, acts on the proton pump without the need for gastric acid activation, is fast absorbed, quickly reaches its maximal blood concentration from the day of dose, and has a substantially longer action duration. It is expected to partially supplement the unmet clinical needs of PPI and showed a noninferior efficacy compared to PPI for the treatment of gastric ulcers and the prevention of peptic ulcers in NSAID users [48–51].

20.7 Complications

The complications of peptic ulcer, including hemorrhage, perforation, penetration, and gastric outlet obstruction, develop in 25% of patients with peptic ulcer [52]. In contrast to the decreasing prevalence of peptic ulcer, the admission rate from complications of peptic ulcer is reported to be constant or increasing [4, 5]. Peptic ulcer hemorrhage is the most frequent complication, and perforation, gastric outlet obstruction, and penetration follow in the order. *H. pylori* infection and NSAID use are also risk factors, and non-*H. pylori* and non-NSAID peptic ulcer are drawing attention for the developing complications of peptic ulcer.

20.7.1 Gastroduodenal Hemorrhage

Upper gastrointestinal hemorrhage is the most frequent complication and develops in 15–20% of patients with peptic ulcer [52, 53]. In contrast to the decreasing trend of complications of organic perforation or gastric outlet obstruction, upper gastrointestinal hemorrhage from peptic ulcer is increasing, mainly due to the wide use of NSAIDs or aspirin in elderly patients [53].

Hematemesis, melena, or hematochezia is the frequent complaint. However, meticulous history taking and physical examination are important because 20% of elderly patients taking NSAIDs showed silent peptic ulcer hemorrhage without definite symptoms [54].

Risk stratification system guides to classify the high rebleeding risk lesions and how to treat the culprit lesions. Blatchford (Table 20.2) or AIMS65 score (Table 20.3), based on laboratory results and clinical features, or Rockall score (Table 20.4) including results of endoscopic examination can be used [9–11]. Forrest classification system (Table 20.5) is the most frequently used tool for the prediction of rebleeding and

Table 20.2 Blatchford scoring system

Parameter	Score value
Blood urea nitrogen (BUN)	
182 mgdL (<6.5 mmol/L)	0
18.2 ≤ BUN <22.4 mg/dL (6.5 ≤ BUN <8 mmol/L)	2
22.4 ≤ BUN <28 mg/dL (8 ≤ BUN <10 mmol/L)	3
28 ≤ BUN <70 mg/dL (10 ≤ BUN <25 mmol/L)	4
70 mg/dL ≤ (25 mmol/L ≤)	6
Hemoglobin (Hb) (male)	
13 g/dL ≤ (130 g/L ≤)	0
12 ≤ Hb <13 g/dL (120 ≤ Hb <130 g/L)	1
10 ≤ Hb <12 g/dL (100 ≤ Hb <120 g/L)	3
Hemoglobin (Hb) (female)	
12 g/dL ≤ (120 g/L ≤)	0
10 ≤ Hb <12 g/dL (100 ≤ Hb <120 g/L)	1
Hemoglobin (Hb) (male or female) <10 g/dL (<100 g/L)	6
Systolic blood pressure (SBP)	
110 mmHg ≤	0
100–109 mmHg	1
90–99 mmHg	2
<90 mmHg	3
Other markers	
Pulse ≥100/min	1
Melena at presentation	1
Syncope at presentation	2
Hepatic disease present	2
Cardiac failure present	2

determination of endoscopic treatments [15]. Gastric lavage with cold saline through nasogastric tube had been used for the diagnosis of active hemorrhage, clearance of endoscopic view, and hemostasis. However, it is no longer recommended because it could delay the endoscopic procedure and diagnostic sensitivity of gastric lavage is low [12–14]. Moreover, endoscopic view can be obtained with changing position of patients [12–14].

Stabilization of vital sign and stopping causative factors, such as NSAIDs, are the first step of treatment. Intravenous PPI is injected for the ulcer healing and prevention of rebleeding.

Table 20.3 AIMS 65 scoring system

Parameter	1 Point for each parameter	Alternative description
Albumin	<3.0 g/dL (30 g/L)	
INR	>1.5	
Mental status	Glasgow coma score <14	Disorientation, lethargy, stupor, or coma
Systolic blood pressure	<90 mmHg	
Age	>65	

Endoscopic hemostasis includes local injection, mechanical hemostasis, thermal coagulation, and local infusion therapy. No single treatment is known to be more effective than the other remaining managements. Combinations of hemostatic treatments are known to be more effective than single treatment in patients with active hemorrhage [16, 17].

The hemostasis success rate is reported to be over 90%, but rebleeding also develops in 10–25% of patients [7]. NSAID discontinuation should be confirmed, and all patients should be tested for the *H. pylori* infection. If *H. pylori* infection was confirmed, the eradication therapy should be followed for the prevention of rebleeding [18]. If impossible to stop the aspirin, combination of PPI with aspirin is recommended [19, 20].

Second-look endoscopic examination, which is performed within 24 h from the first hemostasis, has not shown the beneficial effect in overall mortality and operation rate, although it should be performed when the hemostasis was incomplete or vital sign is unstable [7, 21].

Surgical treatment should be considered when the hemorrhage recurs despite of repeated attempts of endoscopic hemostasis or when the vital sign is unstable [22]. Angiographic embolization showed comparable efficacy to surgery in

Table 20.4 Rockall scoring system

	Score			
Variable	0	1	2	3
Pre-endoscopy				
Age	<60	60–79	≥80	
Shock	No shock with systolic BP ≥100 mmHg and pulse <100/min	Tachycardia (pulse ≥100/min) with systolic BP ≥100 mmHg	Hypotension with systolic BP <100 mmHg	
Comorbidity	No major		Cardiac failure, ischemic heart disease, any major comorbidity	Renal failure, hepatic failure, disseminated malignancy
Postendoscopy				
Diagnosis	Mallory-Weiss tear, no major lesions, no stigmata of recent hemorrhage	All other diagnosis	Upper gastrointestinal tract malignancy	
Major stigmata of recent hemorrhage	None (or dark area only)		Blood in upper gastrointestinal tract (adherent clot, visible or spurting vessel)	

Table 20.5 Forrest classification

Classification and description
Acute hemorrhage
Class Ia—Spurting hemorrhage
Class Ib—Oozing hemorrhage
Signs of recent hemorrhage
Class IIa—Nonbleeding visible vessel
Class IIb—Adherent clot
Class IIc—Flat pigmented spot
Lesions without active bleeding
Class III—Clean ulcer base

the prevention of rebleeding and mortality, and it could be substituted for surgery when the surgical risk of patient is too high [23]. Malignancy should be considered if the ulcer healing is incomplete, and endoscopic biopsy should be performed for this lesion to exclude the malignancy, if it was not massive hemorrhage [6].

20.7.2 Intestinal Perforation

Intestinal perforation occurs in 2–10% of peptic ulcer, and it is the most common cause of operation in patients with peptic ulcer [24]. Anterior wall of duodenal bulb is the most common site (60%), and the antrum (20%) and lesser curvature side of gastric body (20%) follow [6, 12]. However, gastric ulcer perforation is reported to be increasing with the wide use of NSAIDs [6, 12]. Typical symptoms and signs of peritonitis or septic shock could be observed. However, it could be alleviated in elderly, diabetic patients or patients taking steroid, immunosuppressant, and narcotic analgesics [6, 25]. The symptom onset of retroperitoneal perforation is relatively late, and typical signs are not present [26]. History taking and physical examination are important, and detecting free air in X-ray could direct to the diagnosis. However, there is no free air in 10–20% of duodenal ulcer perforation [27, 28]. Therefore, additional study, such as computed tomography, ultrasound, or upper gastrointestinal contrast study, is frequently needed [27, 28].

After making a diagnosis, salvage managements including nasogastric tube drainage, saline infusion, PPI, and wide-spectrum antibiotics are started [6]. Bacterial peritonitis from free perforation is the indication of emergency operation [25]. Surgical methods and resection area could be changed according to the perforation site. If clinical features are stable, nonsurgical management could be considered.

Table 20.6 American Society of Anesthesiologists (ASA) physical status classification system

Classification	Description
ASA 1	Healthy patients
ASA 2	Mild to moderate systemic disease caused by the surgical condition or by other pathological processes and medically well controlled
ASA 3	Severe disease process which limits activity but is not incapacitating
ASA 4	Severe incapacitating disease process that is a constant threat to life
ASA 5	Moribund patient not expected to survive 24 h with or without an operation
ASA 6	Declared brain-dead patient whose organs are being removed for donor purposes

The mortality rate of intestinal perforation from peptic ulcer was reported to be 6–14%, and delay of diagnosis and treatment, comorbidities, NSAID use, and old age (>70 years) were proved to be risk factors associated with mortality [7, 30]. Various risk stratification systems for the prediction of prognosis have been developed. Boey score and ASA score (Table 20.6) are the most frequently used scoring system, but the usefulness of each method is different from study [31].

20.7.3 Gastric Outlet Obstruction

Gastric outlet obstruction refers to the intestinal obstruction of prepyloric area, duodenal bulb, or postbulbar area. Malignancy is the most common etiology taking 66% of all causes, and peptic ulcer is the most common cause among the benign etiologies taking 5–8% of all causes [6, 25, 34]. The mechanism of development is inflammation, edema, and fibrosis from repeated ulceration. Nausea, vomiting, early satiety, and weight loss are frequent complaints.

Endoscopy or contrast study is used for the diagnosis. Malignancy should be excluded before the initiation of treatment. Computed tomography or endoscopic ultrasound can be used for the exclusion of malignancy and measuring the stenotic lesion. Balloon dilatation using the endoscopic instrument is used for the treatment, and only focal stenotic lesions could be managed. For the prevention of restenosis after balloon dilatation, steroid injection can be performed using endoscopic ultrasound [34]. Obstructive lesion should be observed before the therapeutic procedure, and active ulcer should be managed with antiulcer medication or *H. pylori* eradication. Mechanical dilatation without the management of treatable ulcer cannot achieve the satisfactory results [34]. Balloon dilatation is repeated approximately every 1–3 weeks interval. The definite endpoint of treatment is not decided. According to one study, endpoint of 41 Fr. balloon and 15 mm diameter of gastric outlet showed the 80% of immediate symptomatic relief [35]. Graded or stepwise approach is used in every single procedure (8 → 10 mm, 10 → 12 mm, 12 → 15 mm).

Treatment outcome is reported to be various, but the overall long-term therapeutic outcome is estimated over 75%, if *H. pylori* eradication, PPI use, and stopping NSAIDs were accomplished [34]. Cautious interpretation is also needed because some of the studies did not include *H. pylori* eradication or prescription of PPI in the treatment, and various therapeutic indicators, such as symptomatic relief or recurrence of gastric outlet obstruction, were used for the determination of therapeutic efficacy. Short-term efficacy showed satisfactory results, but repeated procedures is usually needed. The development of intestinal perforation is the most serious complication. Patients with hematologic coagulopathy, cardiovascular diseases, or acute stage ulcer that can lead to the perforation are not recommended for the procedure. Nonresponders to medication or patients with recurrent gastric outlet obstruction should be considered for surgical management.

20.7.4 Penetration and Fistula

The penetration from peptic ulcer develops when the ulcer progresses to the direction of organic axis without the development of perforation. The nature, location, and duration of pain change with the development of penetration, and patients with penetration usually have a history of longstanding peptic ulcer [32]. The penetration can be made in the near organs, such as pancreas, liver, biliary tract, colon, or vessels [33]. The penetration made between the gastric antrum to bulb of duodenum and located near the normal pylorus is called double pylorus [33]. The diagnosis is made through the endoscopic or radiologic examination [33]. Treatment includes PPI, antiulcer medications, *H. pylori* eradication, and stopping NSAIDs and surgical managements are only needed in cases with serious conditions.

20.7.5 Prevention of Peptic Ulcer

According to a classification of preventive methods based on the etiology, *H. pylori* eradication is the most efficient way for the treatment and prevention of peptic ulcers [39]. Regarding NSAIDs, it is recommended to investigate the risk factors for peptic ulcers and cardiovascular disease in patients on long-term NSAIDs, and to manage accordingly [55, 56]. High-risk factors of NSAID-induced peptic ulcer and its complications include old age, a history of peptic ulcer, administration of high-dose NSAIDs, and coadministration of aspirin, antiplatelet agent, anticoagulants or steroid [56]. In high-risk groups with multiple risk factors, administration of the NSAIDs should be stopped if possible, and preventive measures for the peptic ulcer should be taken [55].

If NSAIDs must be administered, it is important to administer them at the lowest effective dose for an appropriate period of time. Patients with long-term NSAID or low-dose aspirin therapy are recommended testing for, and treatment of *H. pylori* infection to prevent peptic ulcer and its complications [56, 57]. These patients with high-risk factors are also recommended to receive low-dose PPIs to prevent peptic ulcer and its complications [56, 57].

The risk of cardiovascular diseases should be considered in high-risk patients who are taking NSAIDs, and these patients with low-risk of cardiovascular diseases are recommended to administrate COX-2 inhibitor as NSAID [56, 57].

20.8 Conclusions

Peptic ulcer is decreasing with the use of PPI and decreasing prevalence of *H. pylori* infection. However, complications of peptic ulcer are still common in clinical practice. Early detection and diagnosis with endoscopic and radiologic examination leads to the high treatment success and low recurrence rate. Multidisciplinary approach of treatments including medications, endoscopic procedures, angiographic hemostasis, and surgical managements is important for the treatment of peptic ulcer and its complications. Further studies about non-*H. pylori*, non-NSAID ulcer will be useful for the prevention of complications and recurrence of peptic ulcer.

References

1. Malfertheiner P, Chan FK, McColl KE. Peptic ulcer disease. Lancet. 2009;374:1449–61.
2. Sung JJ, Kuipers EJ, El-Serag HB. Systematic review: the global incidence and prevalence of peptic ulcer disease. Aliment Pharmacol Ther. 2009;29:938–46.
3. Wang AY, Peura DA. The prevalence and incidence of *Helicobacter pylori*-associated peptic ulcer disease and upper gastrointestinal bleeding throughout the world. Gastrointest Endosc Clin N Am. 2011;21:613–35.
4. Manuel D, Cutler A, Goldstein J, Fennerty MB, Brown K. Decreasing prevalence combined with increasing eradication of *Helicobacter pylori* infection in the United States has not resulted in fewer hospital admissions for peptic ulcer disease-related complications. Aliment Pharmacol Ther. 2007;25:1423–7.
5. Post PN, Kuipers EJ, Meijer GA. Declining incidence of peptic ulcer but not of its complications: a nationwide study in The Netherlands. Aliment Pharmacol Ther. 2006;23:1587–93.
6. Peterson WL, Ciociola AA, Sykes DL, McSorley DJ, Webb DD. Ranitidine bismuth citrate plus clarithromycin is effective for healing duodenal ulcers, eradicating *H. pylori* and reducing ulcer recurrence. RBC *H. pylori* Study Group. Aliment Pharmacol Ther. 1996;10:251–61.
7. Kim N, Kim JW, Kim HJ, Chung MG, Park SM, Baik GH, et al. Distribution of upper gastroduodenal diseases in health check-up subjects in 2006. Korean J Helicobacter Upper Gastrointest Res. 2008;8:1–8.
8. Kim JJ, Kim N, Park HK, Jo HJ, Shin CM, Lee SH, et al. Clinical characteristics of patients diagnosed as peptic ulcer disease in the third referral center in 2007. Korean J Gastroenterol. 2012;59:338–46.
9. Kim HS, Baik SJ, Kim KH, Oh CR, Lee JH, Jo WJ, et al. Prevalence of and risk factors for gastrointestinal diseases in Korean Americans and native Koreans undergoing screening endoscopy. Gut Liver. 2013;7:539–45.
10. Kwon JH, Choi MG, Lee SW, Shu XX, Bae SH, Choi JY, et al. Trends of gastrointestinal diseases at a single institution in Korea over the past two decades. Gut Liver. 2009;3:252–8.
11. Kim JI, Kim SG, Kim N, Kim JG, Shin SJ, Kim SW, et al. Changing prevalence of upper gastrointestinal disease in 28 893 Koreans from 1995 to 2005. Eur J Gastroenterol Hepatol. 2009;21:787–93.
12. Jang HJ, Choi MH, Shin WG, Kim KH, Chung YW, Kim KO, et al. Has peptic ulcer disease changed during the past ten years in Korea? A prospective multicenter study. Dig Dis Sci. 2008;53:1527–31.
13. Wong GL, Wong VW, Chan Y, Ching JY, Au K, Hui AJ, et al. High incidence of mortality and recurrent bleeding in patients with *Helicobacter pylori*-negative idiopathic bleeding ulcers. Gastroenterology. 2009;137:525–31.
14. Bytzer P, Teglbjaerg PS. *Helicobacter pylori*-negative duodenal ulcers: prevalence, clinical characteristics, and prognosis—results from a randomized trial with 2-year follow-up. Am J Gastroenterol. 2001;96:1409–16.
15. Yim JY, Kim N, Choi SH, Kim YS, Cho KR, Kim SS, et al. Seroprevalence of *Helicobacter pylori* in South Korea. Helicobacter. 2007;12:333–40.
16. Meucci G, Di Battista R, Abbiati C, Benassi R, Bierti L, Bortoli A, et al. Prevalence and risk factors of *Helicobacter pylori*-negative peptic ulcer: a multicenter study. J Clin Gastroenterol. 2000;31:42–7.
17. Vu C, Ng YY. Prevalence of *Helicobacter pylori* in peptic ulcer disease in a Singapore hospital. Singap Med J. 2000;41:478–81.
18. Kuipers EJ, Thijs JC, Festen HP. The prevalence of *Helicobacter pylori* in peptic ulcer disease. Aliment Pharmacol Ther. 1995;9(Suppl 2):59–69.
19. Ciociola AA, McSorley DJ, Turner K, Sykes D, Palmer JB. *Helicobacter pylori* infection rates in duodenal ulcer patients in the United States may be lower than previously estimated. Am J Gastroenterol. 1999;94:1834–40.

20. Tsuji H, Kohli Y, Fukumitsu S, Morita K, Kaneko H, Ohkawara T, et al. *Helicobacter pylori*-negative gastric and duodenal ulcers. J Gastroenterol. 1999;34:455–60.
21. Borody TJ, George LL, Brandl S, Andrews P, Ostapowicz N, Hyland L, et al. *Helicobacter pylori*-negative duodenal ulcer. Am J Gastroenterol. 1991;86:1154–7.
22. Papatheodoridis GV, Archimandritis AJ. Role of *Helicobacter pylori* eradication in aspirin or non-steroidal anti-inflammatory drug users. World J Gastroenterol. 2005;11:3811–6.
23. Ramsay LE, Sanmuganathan PS, Wallis EJ, Jackson PR. Aspirin for primary prevention. Treatment policy should be based on all trial evidence, not subgroup analysis. BMJ. 2000;321:1472.
24. Musumba C, Pritchard DM, Pirmohamed M. Review article: cellular and molecular mechanisms of NSAID-induced peptic ulcers. Aliment Pharmacol Ther. 2009;30:517–31.
25. Lee JH, Lee YC, Jeon SW, Kim JW, Lee SW. Guidelines of prevention and treatment for NSAID-related peptic ulcers. Korean J Gastroenterol. 2009;54:309–17.
26. Laine L. Approaches to nonsteroidal anti-inflammatory drug use in the high-risk patient. Gastroenterology. 2001;120:594–606.
27. Bhatt DL, Scheiman J, Abraham NS, Antman EM, Chan FK, Furberg CD, et al. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. J Am Coll Cardiol. 2008;52:1502–17.
28. Yoon H, Kim SG, Jung HC, Song IS. High recurrence rate of idiopathic peptic ulcers in long-term follow-up. Gut Liver. 2013;7:175–81.
29. Kang JM, Seo PJ, Kim N, Lee BH, Kwon J, Lee DH, et al. Analysis of direct medical care costs of peptic ulcer disease in a Korean tertiary medical center. Scand J Gastroenterol. 2012;47:36–42.
30. Graham DY, Malaty HM. Alendronate gastric ulcers. Aliment Pharmacol Ther. 1999;13:515–9.
31. Sinar DR, Bozymski EM, Blackshear JL. Effects of oral potassium supplements on upper gastrointestinal mucosa: multicenter clinical comparison of three formulations and placebo. Clin Ther. 1986;8:157–63.
32. Kim DJ, Kim HY, Kim SJ, Hahn TH, Jang MK, Baik GH, et al. *Helicobacter pylori* infection and peptic ulcer disease in patients with liver cirrhosis. Korean J Intern Med. 2008;23:16–21.
33. Jo YW, Choi JY, Ha CY, Min HJ, Lee OJ. The clinical features and prognostic factors of nonvariceal upper gastrointestinal bleeding in the patients with liver cirrhosis. Korean J Helicobacter Upper Gastrointest Res. 2013;13:235–42.
34. Bang CS, Lee YS, Lee YH, Sung H, Park HJ, Kim HS, et al. Characteristics of nonvariceal upper gastrointestinal hemorrhage in patients with chronic kidney disease. World J Gastroenterol. 2013;19:7719–25.
35. Lohr JM, Nelson JA, Oldstone MB. Is herpes simplex virus associated with peptic ulcer disease? J Virol. 1990;64:2168–74.
36. Lu CL, Chang SS, Wang SS, Chang FY, Lee SD. Silent peptic ulcer disease: frequency, factors leading to “silence,” and implications regarding the pathogenesis of visceral symptoms. Gastrointest Endosc. 2004;60:34–8.
37. Rew JS. Classification of ulcer cycle and natural history in gastric ulcer. Korean J Gastrointest Endosc. 2010;41(Suppl 2):15S–8.
38. Choi MH. The endoscopic diagnosis of peptic ulcer disease. Korean J Helicobacter Upper Gastrointest Res. 2014;14:6–12.
39. Cheung DY, Jung HY, Song HJ, Jung SW, Jung HC. Guidelines of treatment for non-bleeding peptic ulcer disease. Korean J Gastroenterol. 2009;54:285–97.
40. Malfertheiner P, Megraud F, O’Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of *Helicobacter pylori* infection—the Maastricht IV/Florence consensus report. Gut. 2012;61:646–64.
41. Scheiman JM. The use of proton pump inhibitors in treating and preventing NSAID-induced mucosal damage. Arthritis Res Ther. 2013;15(Suppl 3):S5.
42. Agrawal NM, Campbell DR, Safdi MA, Lukasik NL, Huang B, Haber MM. Superiority of lansoprazole vs ranitidine in healing nonsteroidal anti-inflammatory drug-associated gastric ulcers: results of a double-blind, randomized, multicenter study. NSAID-Associated Gastric Ulcer Study Group. Arch Intern Med. 2000;160:1455–61.
43. Wu CY, Wu MS, Chen CJ, Li MC, Lin JT, Chen GH. The interaction of *H. pylori* infection and NSAIDs in cyclooxygenase-2 mRNA expression in gastric antral, corpus mucosa, and gastric ulcer. J Clin Gastroenterol. 2005;39:50–5.
44. McColl KE, el-Nujumi AM, Chittajallu RS, Dahill SW, Dorrian CA, el-Omar E, et al. A study of the pathogenesis of *Helicobacter pylori* negative chronic duodenal ulceration. Gut. 1993;34:762–8.
45. Gillen D, Wirz AA, Neithercut WD, Ardill JE, McColl KE. *Helicobacter pylori* infection potentiates the inhibition of gastric acid secretion by omeprazole. Gut. 1999;44:468–75.
46. Oshima T, Miwa H. Potent potassium-competitive acid blockers: a new era for the treatment of acid-related diseases. J Neurogastroenterol Motil. 2018;24:334–44.
47. Inatomi N, Matsukawa J, Sakurai Y, Otake K. Potassium-competitive acid blockers: advanced therapeutic option for acid-related diseases. Pharmacol Ther. 2016;168:12–22.
48. Miwa H, Uedo N, Watari J, Mori Y, Sakurai Y, Takanami Y, et al. Randomised clinical trial: efficacy and safety of vonoprazan vs. lansoprazol in patients

with gastric or duodenal ulcers–results from two phase 3, non-inferiority randomized controlled trials. Aliment Pharmacol Ther. 2017;45:240–52.

49. Cho YK, Choi MG, Choi SC, Lee KM, Kim TO, Park SH, et al. Randomised clinical trial: tegoprazan, a novel potassium-competitive acid blocker, or lansoprazol in the treatment of gastric ulcer. Aliment Pharmacol Ther. 2020;52:789–97.
50. Kawai T, Oda K, Funao N, Nishimura A, Matsumoto Y, Mizokami Y, et al. Vonoprazan prevents low-dose aspirin-associated ulcer recurrence: randomised phase 3 study. Gut. 2018;67:1033–41.
51. Mizokami Y, Oda K, Funao N, Nishimura A, Soen S, Kawai T, et al. Vonoprazan prevents ulcer recurrence during long-term NSAID therapy: randomised, lansoprazol-controlled non-inferiority and single-blind extension study. Gut. 2018;67:1042–51.
52. Ramakrishnan K, Salinas RC. Peptic ulcer disease. Am Fam Physician. 2007;76:1005–12.
53. Milosavljevic T, Kostić-Milosavljević M, Jovanović I, Krstić M. Complications of peptic ulcer disease. Dig Dis. 2011;29:491–3.
54. Martinez JP, Mattu A. Abdominal pain in the elderly. Emerg Med Clin North Am. 2006;24:371–88, vii.
55. Lanza FL, Chan FK, Quigley EM. Practice Parameters Committee of the American College of Gastroenterology. Guidelines for prevention of NSAID-related ulcer complications. Am J Gastroenterol. 2009;104:728–38.
56. Joo MK, Park CH, Kim JS, Park JM, Ahn JY, Lee BE, et al. Clinical guidelines for drug-induced peptic ulcer, 2020 revised edition. Korean J Gastroenterol 76:108–133.
57. Kamada T, Satoh K, Itoh T, Ito M, Iwamoto J, Okimoto T, et al. Evidence-based clinical practice guidelines for peptic ulcer disease 2020. J Gastroenterol. 2021;56:303–22.

21 Gastric Extranodal Marginal Zone B-Cell Lymphoma of MALT

Yoon Jin Choi

Abstract

Low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma occurs worldwide and is mostly found in gastric antrum and gastric body. It is strongly associated with *Helicobacter pylori* (*H. pylori*). *H. pylori* infection rate was reported to be as high as 90%, although the portion of *H. pylori*-negative cases have been increasing. Therefore, diagnosis and treatment of *H. pylori* infection is the first step in the management regardless of the disease stage. For stage I–IIE diseases, complete remission rate has reached approximately 80% following the successful *H. pylori* eradication. Although most remission occurs within 6–12 months after the end of the treatment, it takes more for some cases, to 24 months or more. This indolent characteristic of the low-grade gastric MALT lymphoma has brought about changes in the treatment and follow-up of the disease. As long as there is no overt clinical and histological progression of the disease, even if there are residual lesions histologically, it is possible to wait for the complete remission with an esophagogastroduodenoscopy every 3–6 months. Even in the case of asymptomatic disseminated diseases, if they are stable, chemotherapy can be postponed with monitoring by physical examination, endoscopic study, imaging as clinically required, blood counts and biochemistry every 3–6 months.

Keywords

Helicobacter pylori · Marginal zone B-cell lymphoma · Mucosa-associated lymphoid tissue (MALT) · MALT lymphoma

21.1 Introduction

Primary gastric lymphoma is a rare disease accounting 1–7% of gastric malignant tumors, but accounts 60% among gastrointestinal tract lymphoma. The prevalence of primary gastric lymphoma is increasing compared to the decreasing prevalence of gastric adenocarcinoma. Around 80% of primary gastric lymphoma cases are B-cell non-Hodgkin lymphoma, which is divided by mucosa-associated lymphoid tissue (MALT) lymphoma and high-grade diffuse large cell lymphoma. In 1983, Isaacson and Wright first established the concept of mucosa-associated lymph originating from MALT, and the term MALT lymphoma became widely used since then [1]. The gastrointestinal (GI) tract is the most frequent site of MALT lymphoma, and the stomach is involved in up to two-thirds of these cases. It

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sometimes occurs in the ocular adnexa, lung, urinary tract, thyroids, and gall bladder other than the GI tract. Low-grade gastric MALT lymphoma was revealed to be caused by *Helicobacter pylori* (*H. pylori*) as an immune response on the gastric mucosa. Patients with low-grade gastric MALT lymphoma were reported to have very high *H. pylori* infection rate up to 90%, while the number of *H. pylori*-negative diseases have gradually increased up to 20–30% [2, 3].

Since low-grade gastric MALT lymphomas have nonspecific endoscopic findings, it is often difficult to differentiate from benign diseases. The author, herein, reviewed the clinicopathologic characteristics, diagnosis, treatment, and prognosis.

21.2 Pathogenesis of Gastric MALT Lymphoma

Although primary MALT lymphoma occurs most commonly in the stomach, the stomach usually has no organized lymphoid tissue. However, chronic inflammation can induce organized lymphoid tissues. Gastric MALT lymphoma does homing to mucosa in other organs rather than spreading to lymph nodes. A dissemination in the stomach on a molecular level was noted in the most cases and this was sometimes extended to the bowel mucosa [4].

21.2.1 *H. pylori*

Low-grade MALT lymphoma of the stomach is strongly associated with *H. pylori* infection. Up to 90% of patients with gastric MALT lymphoma are *H. pylori*-positive in the early 2000s [5]. Although there is no lymphoid tissue in normal mucosa, infection with *H. pylori* triggers proliferation of T cells and B cell by antigen presenting eventually resulting in the formation of acquired lymphoid tissues [6]. The bacteria directly contact with gastric epithelial cells, and this promotes to the production of multiple inflammatory cytokines, such as interleukin (IL)-1ß, IL-2, IL-6, IL-8, and tumor necrosis factor (TNF)-α. Among them, it is believed that IL-8 is the most important mediator for the inflammatory reaction in mucosa [7]. Cells in the MALT lymph tissue proliferate leading to lymphoid follicular hyperplasia. Activated cells are transformed into tumor cells and finally result in malignant lymphoma through cytogenetic changes, chromosomal abnormality, and induction of autoimmunological clones or dysregulation of apoptosis. Persistent *H. pylori* infection causes chronic gastritis, then, can develop into more organized gastric MALT with the histological and functional similarities to Peyer's patches in the small intestine [8]. In the earliest stage, the neoplastic cells (sometimes known as centrocyte-like cells) adopt a perifollicular distribution, but the infiltrate extends into the lamina propria away from the follicles and this may be a helpful diagnostic feature. The neoplastic cells infiltrate into gastric gland epithelium causing eosinophilic change to the epithelial cells and destruction of the architecture (lymphoepithelial lesion).

The neoplastic cells have variable morphology including mature round lymphocyte cells resembling germinal center centrocytes with irregular nuclei, cells with monocytoid/marginal zone B-cell appearance, and cells with lymphoplasmacytic appearances. All cases have a variable number of large transformed cells. However, when large neoplastic cells are present in sheets, the diagnosis of an associated diffuse large B-cell lymphoma (DLBCL) should be made. Although there are no definitive criteria, a diagnosis of transformation to large B-cell lymphoma should be made when there are separated sheets of large cells comprising >20% of the neoplastic population.

21.2.2 Genetic Variation

Several genetic changes are implicated in the development of gastric MALT lymphoma. Translocations t(11;18)(q21;q21), t(1;14)(p22;q32), and t(14;18)(q32;q21) are most frequently found in MALT lymphoma [9]. Importantly, nuclear factor kappa B (NF-κB) is known to mediate cell survival and antiapoptotic signals [10], it has been speculated that its upregulation may contribute to the malignant transformation of *H. pylori*-independent growth of MALT lymphomas with refractory to anti-*H. pylori* therapy [11].

Most common genetic variation is t(11;18)(q21;q21), which can be found 25–30% of MALT lymphoma [12]. Translocation t(11;18)(q21;q21) fuses the N-terminus of the *API2* gene to the C-terminus of the *MALT1* gene and generates a functional API2-MALT1 fusion product [11] (Fig. 21.1). The chimeric protein of t(11;18)(q21;q21) itself leads to constitutive NF-κB activity through self-oligomerization of the baculovirus IAP repeat (BIR) domain of the API2 molecule. This translocation is a specific marker for *H. pylori* independence of low-grade gastric MALT lymphoma [13].

Another important genetic aberration, t(1;14)(p22;q32), which is found in less than 5% of MALT lymphomas, juxtaposes the *BCL10* gene of chromosome 1p to the immunoglobulin gene locus of chromosome 14q. This results in strong expression of a truncated BCL10 protein in the nuclei and cytoplasm [14]. BCL10 is an intracellular protein that positively regulates lymphocyte proliferation by linking antigen receptor stimulation to activate NF-κB signaling. Finally, translocation t(14;18)(q32;q21) is only rarely found in gastric MALT lymphoma [15].

Besides these translocations, it has been recently reported that chromosome 6q was frequently deleted in ocular marginal zone B-cell lymphoma and identified *TNFAIP3/A20*, a negative regulator of NF-κB pathways, as the primary target for 6q deletion [16]. However, this has been found in only 7% of gastric MALT lymphoma, and further study about the association of this genetic alteration and gastric MALT lymphoma is needed.

21.3 Clinical Feature and Diagnosis

21.3.1 Clinical Characteristics and Endoscopic Features

Low-grade gastric MALT lymphoma occurs over a wide range of age, but most common from 55 to 60 years old [17]. The sex ratio incidence is essentially equal [17]. It is usually localized on the antrum and body but can occur everywhere in the stomach [18].

The symptoms are absent or vague in most patients with early-stage low-grade lymphoma. Therefore, gastric MALT lymphoma is sometimes diagnosed incidentally following an upper endoscopy performed for regular cancer-screening program. Similarly, various and nonspecific endoscopy findings have been described for the disease. It is frequently characterized by erosions, small nodules, and thickening of gastric folds, generally suggesting a benign condition, or even by normal gastric mucosa.

Although there is no consensus for the endoscopic classification of gastric MALT lymphoma, it can be divided to exophytic type, ulceroinfiltrative type, superficial type, and others [19]. By using the classification of Yokoi et al. [20], superficial lesions could be grouped into six categories: IIc-like type, submucosal tumor type, multiple erosion type, cobblestone-mucosa type, partial-fold swelling type, and discoloration type [18] (Fig. 21.2).

The neoplasia can be detected on normal-appearing mucosa with solely patch discoloration or mild nodularity. Erosions or ulcers in gastric MALT lymphoma often seem irregular or geo-

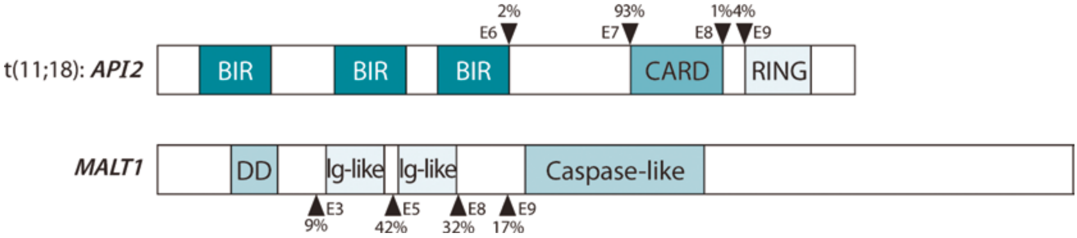

Fig. 21.1 Representation of the *API2* and *MALT1* genes involved in t(11;18). *Vertical arrows* represent the known breakpoints that give rise to the *API2-MALT1* fusion and their incidence in percentages. *BIR* Baculovirus IAP repeat, *CARD* Caspase recruitment domain, *Ring* Really interesting new gene, *DD* Death domain

graphical. It is often present in multiple locations in the stomach, while usual cancers or benign ulcers develop in a centripetal pattern. The homing and disseminating tendency may cause the multifocal distribution of the lesions. The concordance of the first endoscopic impression and histological diagnosis is less than 80% [21]; thus, multiple biopsies of any suspicious lesions should be done for the diagnosis of the disease.

21.3.2 Diagnosis and Pathology

Lymphoepithelial lesion is the important diagnostic feature of gastric MALT lymphoma [22] (Fig. 21.3a, b). However, the lymphoepithelial lesions are neither essential for the diagnosis of gastric MALT lymphoma nor specific for this entity; they can be seen in reactive conditions or in other subtypes of lymphoma [23].

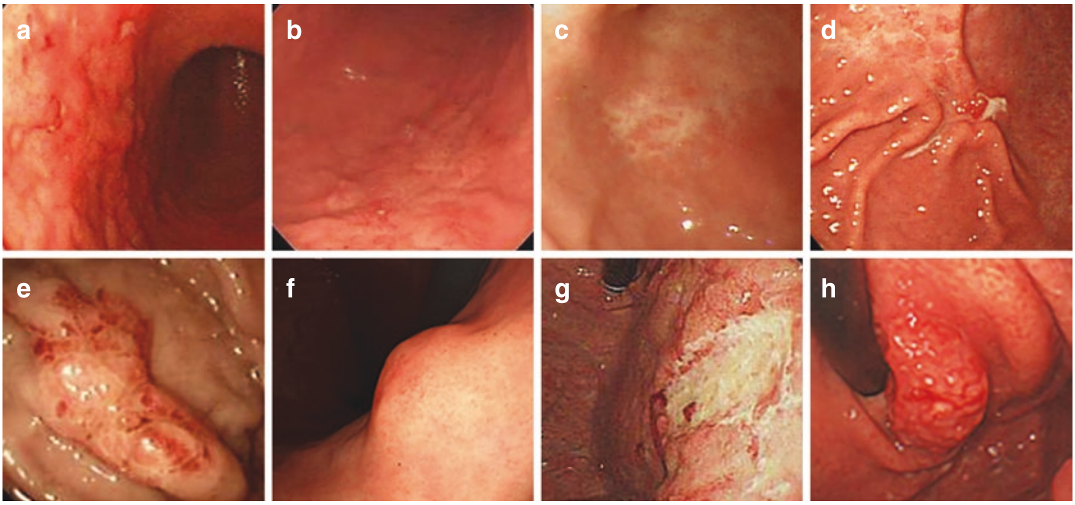

Fig. 21.2 Various endoscopic findings of low-grade gastric MALT lymphoma. (**a**–**f**) Superficial lesions: (**a**) multiple erosion type, (**b**) nodularity (or cobblestone-mucosa type), (**c**) discoloration type, (**d**, **g**) IIc like type, (**e**, **h**) partial-fold swelling, (**f**) submucosal tumor type (Adapted from Choi et al. [18])

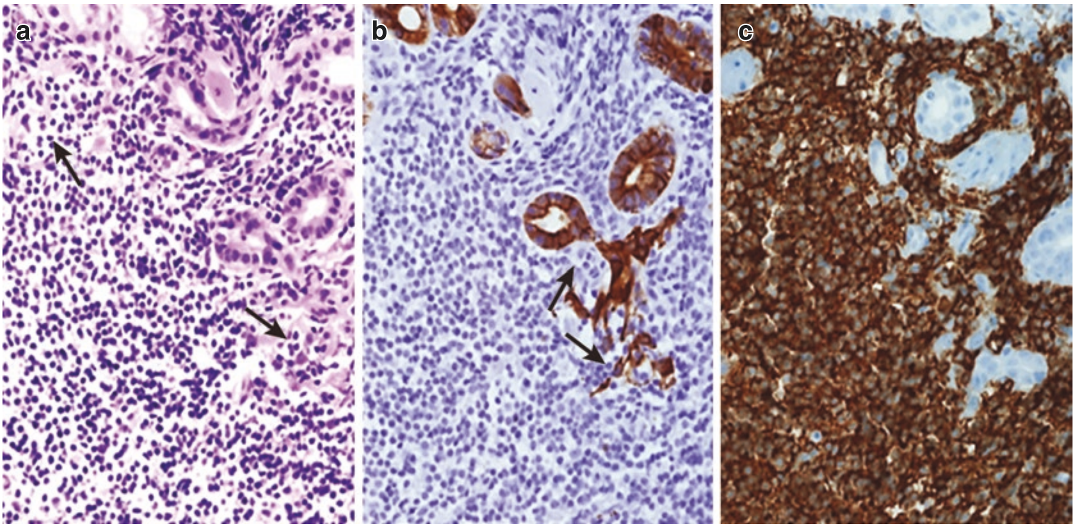

Fig. 21.3 Infiltration patterns of gastric mucosa-associated lymphoid tissue (MALT) lymphoma. (**a**) As a hallmark of gastric MALT lymphoma, lymphoepithelial lesions were detected in H&E staining. (**b**) Lymphoepithelial lesions stained by cytokeratin (*brown*). (**c**) Diffuse stain with pan-B-cell markers CD20 immunohistochemistry (*brown*)

Table 21.1 Histological scoring system for gastric MALT lymphoma (WHO/Wotherspoon score)

Score	Diagnosis	Histological features
0	Normal	Scattered plasma cells in lamina propria. No lymphoid follicles
1	Chronic active gastritis	Small clusters of lymphocytes in lamina propria
		No lymphoid follicles. No lymphoepithelial lesions
2	Chronic active gastritis with florid lymphoid follicle formation	Prominent lymphoid follicles with surrounding mantle zone and plasma cells
		No lymphoepithelial lesions
3	Suspicious lymphoid infiltrate, probably reactive	Lymphoid follicles surrounded by small lymphocytes that infiltrate diffusely in lamina propria and occasionally into epithelium
4	Suspicious lymphoid infiltrate, probably lymphoma	Lymphoid follicles surrounded by marginal zone cells that infiltrate diffusely in lamina propria and into epithelium in small groups
5	MALT lymphoma	Presence of dense infiltrate of marginal zone cells in lamina propria with prominent lymphoepithelial lesions

Adapted from Wotherspoon et al. [25]

Immunophenotypically, these cells express pan-B-cell markers, CD20, while they show no immunoreactivity for CD5, CD10, and Cyclin D1 (Fig. 21.3c). Staining for Ki67 may help in identifying large cell components [24]. There is a widely accepted consensus that the diagnosis of gastric MALT lymphoma is based on histomorphological criteria according to the WHO classification [25] (Table 21.1).

The histological criteria for the diagnosis of gastric MALT lymphoma are using the 5-grade system (Table 21.1) which Wotherspoon et al. reported [25]. Based on the system, the lesion with a score of 5 is defined as MALT lymphoma, but lesions with a score of 3 or 4 are defined as MALT lymphoma when the monoclonality is demonstrated.

21.3.3 Staging

Although gastric MALT lymphoma has an indolent course with a low morbidity and mortality, an accurate staging procedure is mandatory.

Recent studies report that about 20–25% of patients with gastric MALT lymphoma have clinical dissemination at extensive staging, whereas the risk of patients with extragastric origin reaches 40–50%.

Initial workup for staging should include a complete physical examination including lymph node regions, eyes and ears, nose and throat, liver and spleen, routine laboratory tests (full blood and differential counts, lactate dehydrogenase, and β2-microglobulin), computed tomography of the chest, abdomen, and pelvis.

The endoscopic ultrasound (EUS) can be used to assess both the infiltration of lymphoma in the gastric wall and the regional lymph nodes involvement [26]. The depth of invasion into the different layers of gastric walls is a predictive factor of lymphoma remission following the therapy.

PET scanning should also be considered when clinical and/or laboratory data suggest transformation to high-grade histology and to guide decisions on which lymph node to target for biopsy.

Bone marrow involvement (stage IV) has been reported in up to almost 10%. However, bone marrow biopsy is no longer mandatory in the overall gastric MALT lymphoma. It is recommended only in special situation in extra gastric MALT lymphomas [23].

The Lugano staging system that has been widely used [23] is still used (Table 21.2), but the clinical relevance of TNM stage has not been validated.

Table 21.2 Comparison of the different staging systems for gastric MALT lymphoma

Lugano system	Modified Ann Arbor system	Paris staging system	Tumor extension
I Confined to GI tract (single primary or multiple, noncontiguous)			
I1: Mucosa, submucosa I2: Muscularis propria, serosa	I_E I_E	T1 N0 M0 T2–3 N0 M0	Mucosa, submucosa Muscularis propria, serosa
II Extending into abdomen			
II1: Regional nodal involvement II2: Distant nodal involvement	II_E II_E	T1–3 N1 M0 T1–3 N2 M0	Perigastric lymph nodes Distant regional lymph nodes
IIE Penetration of serosa to involve adjacent organs or tissues	II_E	T4 N0–2 M0	Invasion of adjacent structures
IV Disseminated or diffuse infiltration of distant or extra-GI organs	III_E IV	T1–4 N3M0 T1–4 N0–3 M1	Lymph nodes on both sides of the diaphragm or distant metastases

Adapted from Zucca et al. [23]
MALT lymphoma Mucosa-associated lymphoid tissue lymphoma, *GI* Gastrointestinal

21.4 Therapeutic Options

Patients with *H. pylori*-associated gastric MALT lymphoma should be given antibiotics regardless of stage. Therefore, diagnosis and treatment of *H. pylori* infection is the first step in the management of gastric MALT lymphoma [24]. If the presence of active *H. pylori* infection is not detected by immunohistochemistry, it must be ruled out by serology, urea breath test, and/or stool antigen test [23].

21.4.1 Anti-*H. pylori* Therapy

H. pylori eradication therapy should be given to all patients with gastric MALT lymphoma, irrespective of stage even in the *H. pylori*-negative patients.

21.4.1.1 Indication and the Effect

For stage I–IIE *H.pylori*-positive diseases, *H. pylori* eradication is the initial treatment of choice, with a curative intent. Regimens for the anti-*H. pylori* therapy can be chosen according to regional resistance profile.

Approximately 80% of patients with stage I–IIE diseases achieve a complete remission after successful *H. pylori* eradication. Although the time it takes to achieve remission after treatment can vary from a few weeks to 2 years or more, most of case get remission within 1 year [27].

Therefore, for patients who achieve endoscopic remission by *H. pylori* eradication but histologically persisting minimal lesions, it is recommended to wait at least 12 months before switching to another treatment [23].

21.4.1.2 The Predictive Factors for Regression After Anti-*H. pylori* Therapy

Different predictive factors of lymphoma remission have been found, including stage, depth of penetration in the gastric wall, *API2-MALT1* translocation status, or different ethnic. Complete regression of lymphoma was more often observed in stage I than in stage II tumors (78.4% vs. 55.6%) and was more common in mucosal and submucosal lesion than in lesions beyond the submucosal layer [5]. The presence of *API2-MALT1* translocation in lymphoma cells reduces a patient's probability of remission to less than 25% [5]. Having t(11;18)(q21;q21) was a predictive factor for nonresponsiveness for *H. pylori* eradication in our previous study [28]. Remission after eradication of *H. pylori* is more common in Asian than in European populations (84% vs. 55%). In summary, when the lesion is stage IE1 without the translocation of t(11;18)(q21;q21), more than 80% of probability of remission after eradication of *H. pylori* could be expected.

21.4.1.3 Assessment of the Response to Treatment

It takes time for the neoplasia to disappear and be replaced with normal mucosa. There were no definite consensus about how often the patients with gastric MALT lymphoma undergo upper gastrointestinal endoscopy after anti-*H. pylori* therapy. The remission is usually achieved 3–12 months after termination of anti-*H. pylori* therapy. Recently, European society for medical oncology recommended that the first follow-up endoscopy could be done 3–6 months after termination of the anti-*H. pylori* treatment, and it can be repeated every 3–6 months until the remission is demonstrated [23].

Same as the initial diagnosis, sequential evaluation of gastric biopsies is fundamental follow-up procedure. In the case of advanced diseases, watch-and-wait should be monitored by physical examination, imaging as clinically required, blood counts, and biochemistry with endoscopic biopsies. It is recommended that tests are performed every 3–6 months by complete remission.

In order to standardize the histological criteria for evaluating lymphoma response to therapy, pathologists from the Groupe d'Etude des Lymphomes de l'Adulte (GELA) established an ad hoc histological grading system, named the GELA score [29]. Based on these criteria, the posttreatment lesions were divided into four categories: complete histological remission, probable minimal residual disease, responding residual disease, and no change [29] (Table 21.3). Therefore, careful biopsies both in the mucosa where the lesion used to be and in surrounding normal mucosa are still needed during the follow-up endoscopy in patients with endoscopic remission.

21.4.1.4 Long-Term Outcome after Successful Eradication

Outcome studies have demonstrated that *H. pylori* eradication also provides a favorable long-term outcome, with a genuine chance of cure or at least of long-lasting complete remission in the majority of patients. A study with 420 patients with low-grade gastric MALT lymphoma who had undergone successful *H. pylori* eradication and been followed up was published in Japan in 2011 [30].

The mean follow-up duration was 6.5 years. When the characteristics of subjects were analyzed, 90% of patients had clinical stage I disease.

Among 341 patients who underwent EUS, the depth of lymphoma infiltration in the gastric wall was mucosa in 52%, submucosa in 38%, and muscularis propria or beyond in 10% of cases.

Table 21.3 GELA histological grading system for posttreatment evaluation of gastric MALT lymphoma

Category	Lymphoid infiltrate	LEL	Stromal changes
Complete histological remission (ChR)	Absent/almost absent	Absent	Normal
Probable minimal residual disease (pMRD)	Aggregates of lymphoid cells in the LP/MM/SM	Absent	Regression
Responding residual disease (rRD)	Dense, diffuse, or nodular	Focal LEL/absent	Regression
	Extending around glands in LP		
No change (NC)	Dense, diffuse, or nodular	Present, "may be absent"	No change

Adapted from Copie-Bergman et al. [29]

MM Muscularis mucosa, *LP* Lamina propria, *SM* Submucosa, *LEL* Lymphoepithelial lesions

Translocation of t(11;18)/*API2-MALT1* was positive in 30 of 206 patients (15%) who were examined [30]. As a result, 323 patients (77%) responded to *H. pylori* eradication, and the median period from the end of treatment to remission was 4 months. Probabilities of freedom from treatment failure, overall survival, and event-free survival after 10 years were 90%, 95%, and 86%, respectively. Among finally determined 323 responders (complete histological remission and probable minimal residual disease; ChR/pMRD), lymphoma relapse was observed in 10 patients (3.1%), and the time duration from ChR/pMRD varied from 1 to 131 months. Only one patient showed transformation into DLBCL.

According to a recent multicenter study of 1163 gastric MALT lymphoma patients (97.6%, stage IE) in South Korea, 10-year overall survival was 99.1% [31]. The prognosis of low-grade gastric MALT lymphoma was excellent even at advanced stages.

21.4.1.5 Histological Residual Disease

Patients with persistent residual lymphoma infiltrates on histology (probable minimal residual disease or responding residual disease) were usually referred for radiation. However, current guidelines recommend patients who respond partially to first-line *H. pylori* eradication can be managed by a watch-and-wait strategy with regular endoscopies and biopsies [23].

There has been accumulating evidence that patients with minimal residual disease can also have a favorable prognosis. According to a large series of 108 patients with a stage I gastric MALT lymphoma, 102 patients (94%) had a favorable disease course. Of them, 35 (32%) achieved complete remission and 67 (62%) with the minimal histological residual diseases remained stable without changes. Local lymphoma progression was seen in four patients (5%), and one patient developed a DLBCL.

Above-mentioned (21.4.1.4) Japanese long-term follow-up study also reported same results [30]. Fourteen nonresponders without progressive disease have been followed up as a "watch-and-wait" strategy since they rejected further rescue treatment. During the follow-up, any of them have not showed a progressive disease. The other two patients with relapse after the eradication of *H. pylori* achieved the second remission without additional therapy 1 year after the relapse. It took more than 2 years for the 10 responders to achieve the remission after the anti-*H. pylori* therapy.

A recent European consensus report recommends that patients with responding residual disease or histological relapse can be followed for at least 12 months by a "watch-and-wait" strategy [23]. It seems that "watch-and-wait" can be prolonged for more than 2 years unless there is no progression of disease or histological transformation. The decision to continue a "watch-and-wait" strategy or to start oncological treatment should be made comprehensively considering multiple factors including *H. pylori* status, clinical stage, presence of t(11;18), or patients' preference.

21.4.2 Nonresponder to Anti-*H. pylori* Therapy

Patients revealing no change or progression of lymphoma despite successful *H. pylori* eradication be referred for radiation or chemotherapy. Both radiation and chemotherapy are considered as curable modalities for localized gastric MALT lymphoma [24].

21.4.2.1 Radiotherapy

MALT lymphomas have been reported to be highly sensitive to radiotherapy. Unlike other mucosal tumor, most of low-grade MALT lymphomas are infiltrated within the stomach wall, and metastasis of lymph node is also confined in the perigastric area [32]. This characteristic of gastric MALT lymphoma allows the radiotherapy to be a curative option for the patients in stages I and II.

For cases of stage IE and IIE1 MALT lymphoma, 5-year event-free survival rate reached

80–90% by 36–45 Gy of the radiation [33, 34]. Moreover, radiation doses have been reduced over the past few decades. Modest dose (30.6 Gy) of radiation is highly efficacious and has fewer complications [35]. Since side effects of irradiation are strongly dependent on the radiation field and dose, efforts to reduce radiation doses continued. The most recent data have reported that the optimal radiotherapy dose for indolent lymphoma is 24 Gy in 12 fractions [36]. International Lymphoma Radiation Oncology Group recommended dose is 24 Gy, but a low-dose schedule with 4 Gy may also be considered in the elderly.

21.4.2.2 Chemotherapy

Chemotherapy with or without immunotherapy should be given to patients in case of systemic symptom or histological transformation. In other words, if there is systemic symptom nor overt progression, a watch-and-wait stratergy can be done without additional treatment [23].

Chemotherapy, immunotherapy, or combination chemoimmunotherapy are all effective in patients who require systemic treatment. The response rates of chemotherapy with various agents reach almost 100%. There is currently no generally accepted standard chemotherapy in gastric MALT lymphoma. The rituximab with cyclophosphamide, vincristine, and prednisone (COP); combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP); bendamustine; and fludarabine plus cyclophosphamide are possible choices [24]. The clinical effectiveness of combination therapy of rituximab/chlorambucil, rituximab monotherapy, and the combination of rituximab and bendamustine have been proved in clinical studies.

Figure 21.4 summarizes the treatment recommendations based on the European society of medical oncology guidline of 2020 [23].

21.4.3 *H. pylori*-Negative Gastric MALT Lymphoma

An initial therapeutic option with *H. pylori* eradication therapy could be attempted even in *H. pylori*-negative patients.

Occasional lymphoma remission following eradication therapy in some uninfected patients has been observed. A meta-analysis, including data of 25 studies with 110 patients with low-

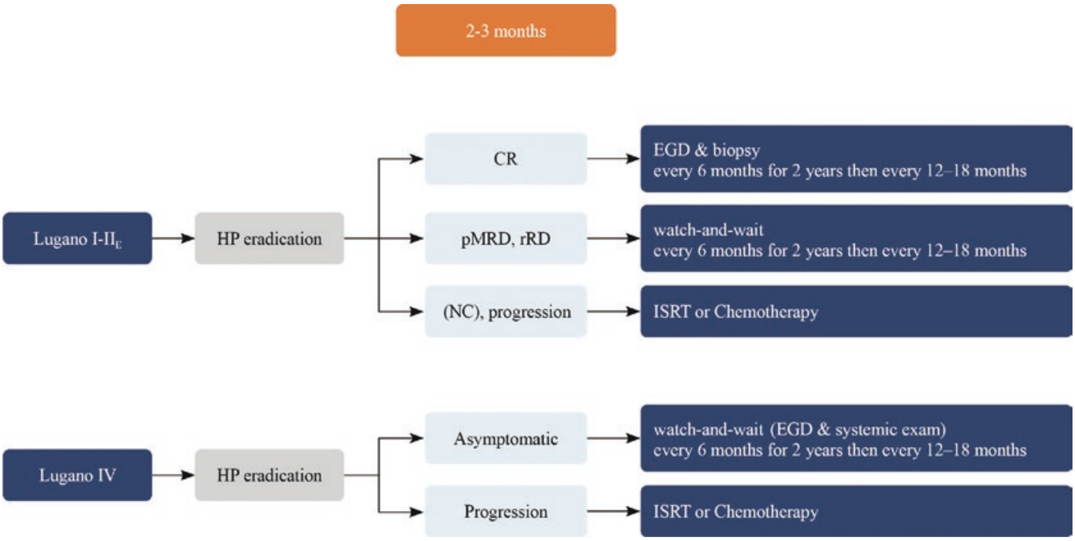
2-3 months
Lugano I-II$_E$
HP eradication
CR
pMRD, rRD
(NC), progression
EGD & biopsy
every 6 months for 2 years then every 12–18 months
watch-and-wait
every 6 months for 2 years then every 12–18 months
ISRT or Chemotherapy
Lugano IV
HP eradication
Asymptomatic
Progression
watch-and-wait (EGD & systemic exam)
every 6 months for 2 years then every 12–18 months
ISRT or Chemotherapy

Fig. 21.4 Therapeutic options of gastric MALT lymphoma. *CR* complete remission, *CTx* chemotherapy, *Hp H. pylori, NC* No change, *pMRD* Probable minimal residual disease, *rRD* Responding residual disease, *RTx* Radiotherapy. Independent of *H. pylori* status (*asterisk*) (Adapted from Zucca et al. [23])

grade gastric MALT lymphoma, showed that overall pooled complete remission rate was 29.3% (95% confidence interval [CI], 22.2–37.4%; $I^2 = 41.5\%$) [37].

However, this could result from a false-negative test or infection by other *Helicobacter* species. Since *H. pylori*-negative cases are less likely to reach remission of the lymphoma after antibiotic therapy, the immediate start of radiotherapy can be considered.

21.5 Surveillance After Remission

Once patients having achieved complete remission after *H. pylori* eradication, esophagoduodenoscopy with biopsy should be repeated every 6 months for 2 years, then every 12–18 months. Surveillance should continue during life [23, 38].

The rationale for the lifelong surveillance is to detect local relapse of MALT lymphoma relatively early; this occurs in about 7% of cases [5]. Moreover, an elevated risk for gastric carcinoma has been reported for patients with MALT lymphoma [39, 40]. In a prospective multicenter trial published recently, there was a significantly higher incidence of gastric cancer (standardized morbidity ratio 8.6; 95% CI, 3.6–20.6) or non-Hodgkin lymphoma (standardized morbidity ratio 18.6; 95% CI, 8.4–41.5) compared with the general population [40].

21.6 Recurrent Disease

Transient histological recurrence is often observed during follow-up. If it persists and progresses, it is considered a relapse. However, this recurrence is often self-limiting, especially in the absence of *H. pylori* reinfection. Therefore, asymptomatic patients with localized relapse can be managed by a watch-and-wait. Otherwise, radiotherapy can be considered to localized diseases. If reinfection is confirmed, antibiotic therapy can be given again.

The recent ESMO guidelines recommend the combination rituximab/lenalidomide as a potential treatment option for relapsed or refractory MALT lymphoma [23].

21.7 Conclusions

H. pylori eradication therapy should be given to all patients with low-grade gastric MALT lymphoma, irrespective of stage, even in *H. pylori*-negative cases. Approximately, 80% of patients with stage I–IIE diseases achieve a complete remission and long-term disease control through only successful *H. pylori* eradication. The indolent nature of low-grade gastric MALT lymphoma and excellent prognosis even in stage IV disease have changed the treatment algorithm; patients with successful *H. pylori* eradication but persisting histological infiltrates can no longer be regarded as treatment failures and regular candidates for radiation or chemotherapy. Instead, patients (even with stage IV disease) can adhere to a watch-and-wait strategy unless there is overt progression, transformation to DLBCL or symptoms. However, in the case of no change or disease progression after anti-*H. pylori* treatment, further treatment should be recommended (radiotherapy for localized diseases; chemotherapy for disseminated diseases). Lifelong surveillance is recommended because of the possibility of recurrence and an increased risk for gastric cancer.

Treatment of gastric MALT lymphoma is still changing. As regular endoscopic assessment with biopsy is essential for the follow-up, a consensus about standardized evaluation form on the endoscopic changes and biopsy technics—including location or number—are needed.

References

1. Isaacson P, Wright DH. Malignant lymphoma of mucosa-associated lymphoid tissue. A distinctive type of B-cell lymphoma. Cancer. 1983;52:1410–6.
2. Asano N, Iijima K, Koike T, Imatani A, Shimosegawa T. *Helicobacter pylori*-negative gastric mucosa-associated lymphoid tissue lymphomas: a review. World J Gastroenterol. 2015;21:8014–20.
3. Takigawa H, Yuge R, Masaki S, Otani R, Kadota H, Naito T, et al. Involvement of non-*Helicobacter pylori* helicobacter infections in *Helicobacter pylori*-negative gastric MALT lymphoma pathogenesis and efficacy of eradication therapy. Gastric Cancer. 2021;24:937–45.
4. Du MQ, Diss TC, Dogan A, Ye HT, Aiello A, Wotherspoon AC, et al. Clone-specific PCR reveals

wide dissemination of gastric MALT lymphoma to the gastric mucosa. J Pathol. 2000;192:488–93.

5. Zullo A, Hassan C, Cristofari F, Andriani A, De Francesco V, Ierardi E, et al. Effects of *Helicobacter pylori* eradication on early stage gastric mucosa–associated lymphoid tissue lymphoma. Clin Gastroenterol Hepatol. 2010;8:105–10.
6. Craig VJ, Arnold I, Gerke C, Huynh MQ, Wündisch T, Neubauer A, et al. Gastric MALT lymphoma B cells express polyreactive, somatically mutated immunoglobulins. Blood. 2010;115:581–91.
7. Peek RM. Pathogenesis of *Helicobacter pylori* infection. Springer Semin Immun. 2005;27:197–215.
8. Isaacson P. The MALT lymphoma concept updated. Ann Oncol. 1995;6:319–20.
9. Streubel B, Simonitsch-Klupp I, Müllauer L, Lamprecht A, Huber D, Siebert R, et al. Variable frequencies of MALT lymphoma-associated genetic aberrations in MALT lymphomas of different sites. Leukemia. 2004;18:1722–6.
10. Farinha P, Gascoyne RD. Molecular pathogenesis of mucosa-associated lymphoid tissue lymphoma. J Clin Oncol. 2005;23:6370–8.
11. Du MQ. MALT lymphoma: many roads lead to nuclear factor-κb activation. Histopathology. 2011;58:26–38.
12. Nakamura S, Matsumoto T. *Helicobacter pylori* and gastric mucosa-associated lymphoid tissue lymphoma: recent progress in pathogenesis and management. World J Gastroenterol. 2013;19:8181.
13. Nakamura S, Ye H, Bacon CM, Goatly A, Liu H, Banham AH, et al. Clinical impact of genetic aberrations in gastric MALT lymphoma: a comprehensive analysis using interphase fluorescence in situ hybridisation. Gut. 2007;56:1358–63.
14. Li Z, Wang H, Xue L, Shin D-M, Roopenian D, Xu W, et al. Eμ-BCL10 mice exhibit constitutive activation of both canonical and noncanonical NF-κB pathways generating marginal zone (MZ) B-cell expansion as a precursor to splenic MZ lymphoma. Blood. 2009;114:4158–68.
15. Streubel B, Lamprecht A, Dierlamm J, Cerroni L, Stolte M, Ott G, et al. T (14; 18)(q32; q21) involving IGH and MALT1 is a frequent chromosomal aberration in MALT lymphoma. Blood. 2003;101:2335–9.
16. Honma K, Tsuzuki S, Nakagawa M, Tagawa H, Nakamura S, Morishima Y, et al. TNFAIP3/A20 functions as a novel tumor suppressor gene in several subtypes of non-Hodgkin lymphomas. Blood. 2009;114:2467–75.
17. Zucca E, Bertoni F, Roggero E, Cavalli F. The gastric marginal zone B-cell lymphoma of MALT type. Blood. 2000;96:410–9.
18. Choi MK, Kim GH. Diagnosis and treatment of gastric MALT lymphoma. Korean J Gastroenterol. 2011;57:272–80.
19. No JH, Lee IS. Endoscopic findings and follow-up of gastric MALT lymphoma. Korean J Med. 2012;83:699–705.
20. Yokoi T, Nakamura T, Nakamura S. Differential diagnosis of gastric MALT lymphomas. Stomach Intest. 2001;36:13–20.
21. Taal B, Boot H, Van Heerde P, De Jong D, Hart A, Burgers J. Primary non-Hodgkin lymphoma of the stomach: endoscopic pattern and prognosis in low versus high grade malignancy in relation to the MALT concept. Gut. 1996;39:556–61.
22. Isaacson PG, Du MQ. Gastrointestinal lymphoma: where morphology meets molecular biology. J Pathol. 2005;205:255–74.
23. Zucca E, Arcaini L, Buske C, Johnson P, Ponzoni M, Raderer M, et al. Marginal zone lymphomas: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2020;31:17–29.
24. Ruskoné-Fourmestraux A, Fischbach W, Aleman B, Boot H, Du M, Megraud F, et al. EGILS consensus report. Gastric extranodal marginal zone B-cell lymphoma of MALT. Gut. 2011;60:747–58.
25. Wotherspoon AC, Diss T, Pan L, Isaacson P, Doglioni C, Moschini A, et al. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet. 1993;342:575–7.
26. El-Zahabi LM, Jamali FR, El H II, Naja M, Salem Z, Shamseddine A, et al. The value of EUS in predicting the response of gastric mucosa-associated lymphoid tissue lymphoma to *Helicobacter pylori* eradication. Gastrointest Endosc. 2007;65:89–96.
27. Gong EJ, Ahn JY, Jung H-Y, Jung K, Cho CJ, Na HK, et al. Bone marrow involvement is not associated with the clinical outcomes of gastric mucosa-associated lymphoid tissue lymphoma. Scand J Gastroenterol. 2016;51:942–8.
28. Choi YJ, Kim N, Paik JH, Kim JM, Lee SH, Park YS, et al. Characteristics of *Helicobacter pylori*-positive and *Helicobacter pylori*-negative gastric mucosa-associated lymphoid tissue lymphoma and their influence on clinical outcome. Helicobacter. 2013;18:197–205.
29. Copie-Bergman C, Gaulard P, Lavergne-Slove A, Brousse N, Fléjou J, Dordonne K, et al. Proposal for a new histological grading system for post-treatment evaluation of gastric MALT lymphoma. Gut. 2003;52:1656.
30. Nakamura S, Sugiyama T, Matsumoto T, Iijima K, Ono S, Tajika M, et al. Long-term clinical outcome of gastric MALT lymphoma after eradication of *Helicobacter pylori*: a multicentre cohort follow-up study of 420 patients in Japan. Gut. 2012;61:507–13.
31. Kim JS, Park JC, Lee JY, Ahn JY, Kang SH, Yang H-J, et al. Long-term clinical outcomes of gastric MALT lymphoma: a nationwide multicenter study in Korea. Front Oncol. 2021;11:681689.
32. Stolte M, Bayerdörffer E, Morgner A, Alpen B, Wündisch T, Thiede C, et al. Helicobacter and gastric MALT lymphoma. Gut. 2002;50:iii19–24.
33. Willich NA, Reinartz G, Horst EJ, Delker G, Reers B, Hiddemann W, et al. Operative and conservative management of primary gastric lymphoma: interim results of a German multicenter study. Int J Radiat Oncol Biol Phys. 2000;46:895–901.
34. Koch P, del Valle F, Berdel WE, Willich NA, Reers B, Hiddemann W, et al. Primary gastrointestinal

non-Hodgkin's lymphoma: II. Combined surgical and conservative or conservative management only in localized gastric lymphoma—results of the prospective German multicenter study GIT NHL 01/92. J Clin Oncol. 2001;19:3874–83.

35. Son SH, Choi BO, Kim GW, Yang SW, Hong YS, Choi IB, et al. Primary radiation therapy in patients with localized orbital marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). Int J Radiat Oncol Biol Phys. 2010;77:86–91.
36. Hoskin P, Popova B, Schofield O, Brammer C, Robinson M, Brunt AM, et al. 4 Gy versus 24 Gy radiotherapy for follicular and marginal zone lymphoma (FoRT): long-term follow-up of a multicentre, randomised, phase 3, non-inferiority trial. Lancet Oncol. 2021;22:332–40.
37. Jung K, Kim DH, Seo HI, Gong EJ, Bang CS. Efficacy of eradication therapy in *Helicobacter pylori*-negative gastric mucosa-associated lymphoid tissue lymphoma: a meta-analysis. Helicobacter. 2021;26:e12774.
38. Zullo A, Hassan C, Andriani A, Cristofari F, De Francesco V, Ierardi E, et al. Eradication therapy for *Helicobacter pylori* in patients with gastric MALT lymphoma: a pooled data analysis. Am J Gastroenterol. 2009;104:1932–7.
39. Capelle LG, de Vries AC, Looman CW, Casparie MK, Boot H, Meijer GA, et al. Gastric MALT lymphoma: epidemiology and high adenocarcinoma risk in a nation-wide study. Eur J Cancer. 2008;44:2470–6.
40. Wündisch T, Dieckhoff P, Greene B, Thiede C, Wilhelm C, Stolte M, et al. Second cancers and residual disease in patients treated for gastric mucosa-associated lymphoid tissue lymphoma by *Helicobacter pylori* eradication and followed for 10 years. Gastroenterology. 2012;143:936–42.

22

Gastric Cancer: Synopsis and Epidemiology of Gastric Cancer

Ernst J. Kuipers

Abstract

Gastric cancer (GC) is the fifth most common cancer accounting for close to 7% of all human cancers. Despite the decrease in incidence, GC remains the most common cause of gastrointestinal cancer-related death, with more than 800,000 fatalities annually. The disease is more common in men than in women, and noncardia GC is twice as common as cardia cancer. More than two-thirds of GC occur in East Asia, in particular, in China, Japan, and Korea. There are large regional and racial differences in the incidence of GC. These differences are related to prevalence of *Helicobacter pylori* (*H. pylori*), diet, and other risk factors. The mortality of GC closely matches the regional differences in incidence. The age-standardized incidence and mortality rates of GC are expected to further decrease due to improvement in socioeconomic conditions and decreasing prevalence of *H. pylori*. Population screening and intervention, as well as general health measures such as antismoking campaigns, can accelerate the changing epidemiology of GC. In the absence of such measures, GC will for long remain a very common and lethal disease. This chapter reviews the epidemiology of GC, with focus on regional differences in incidence and mortality, risk factors for GC. It further summarizes the changing epidemiology of GC in recent decades and the expected future trends.

Keywords

Gastric cancer · Epidemiology · Incidence · Mortality

22.1 Introduction

The first cancer registries started in the late nineteenth century among others in Germany. At that time, 30–40% of all cancers were of gastric origin [1]. That vast predominance of gastric cancer (GC) was strongly related to the ubiquitous colonization of *Helicobacter pylori* (*H. pylori*) from early childhood onwards. Similar findings were observed elsewhere. For instance in the 1930s, GC was almost twice as common as cause of cancer-related death than any other individual malignancy [2]. Since then, we have seen significant changes that are still ongoing. Between 1990 and 2014, the average life expectancy of the world population increased by 6.3 years [3]. This change was most marked in Southeast Asia where life expectancy increased by more than 8 years. These improvements were firstly due to prevention and improved

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outcome of infectious diseases, among others as a result of further implementation of vaccination programs and clean water supply. Other marked improvements were noted in the outcome of treatment of patients with cancer. Both these factors have changed the epidemiology of GC.

22.2 Incidence and Mortality

22.2.1 Incidence

The incidence of noncardia GC has in Western countries continuously decreased over the past 80 years; this decrease was more than 80% in the United States [2]. In Asia, these changes occurred later and were so far less pronounced [4, 5]. GC remained the most common malignancy worldwide until 1975. Nowadays, GC is the fifth most common cancer after cancers of the lung, breast, prostate, and colorectum, with exclusion of nonmelanoma skin cancer, making up for 5.6% of all human cancers [6]. The annual incidence worldwide approximates 1,089,000 cases [6]. This includes 298,000 patients with cardia GC, and 791,000 with noncardia GC. These match age-standardized incidence rates of, respectively, 3.3 and 8.8 per 100,000 worldwide. It is estimated that the incidence of GC will continue to rise to 1.77 million by 2040 [7]. More than two-thirds of GC occur in East Asia, in particular, in China, Japan, and Korea [4] (Fig. 22.1). In 167 (91%) of 184 countries for which there are data, noncardia is more common than cardia GC with an average ratio of 2:1. The few countries in which the incidence of cardia GC is higher than or equal to noncardia cancer can, in particular, be found in Northwestern Europe and include Denmark, Finland, and Sweden. These countries are characterized by high socioeconomic status, low *H. pylori* prevalence, and relatively low proportion of the population consisting of first- and second-generation immigrants. In other Western countries such as the United States and Canada, the incidence of noncardia GC is only marginally higher than that of cardia GC. This pattern markedly differs from that in most Asian and South American countries where noncardia GC tends to be considerably more common than cardia GC. In Eastern and Southeastern Asia, for instance, the age-standardized incidence rates for cardia and noncardia GC approximate 9 and 22 cases per 100,000 per year [7]. A similar pattern existed in most Western countries until recently. Within individual countries, marked differences in the incidence of GC are often observed. One consistent observation is the considerably higher incidence in various indigenous populations [8]. These differences are observed in many different countries with indigenous populations often suffering from an at least fourfold increased standardized incidence ratio of GC compared with the national average. Other groups also differ in the incidence of GC. In the United States, for instance, the incidence of noncardia GC is considerably higher among African-Americans and Hispanics than among Caucasians [9]. In other countries, these differences among population subgroups can even be larger. In Malaysia, for instance, the incidence is more than fivefold higher among Chinese and Indian men and women than among Malay men and women [10].

The highest incidences of both cardia GC are found in China, Central Asia, parts of Eastern Europe and Baltic countries, Central America, and parts of South America [7] (Fig. 22.1). The lowest incidences are observed in the Sahel region and sub-Saharan Africa. The pattern for noncardia GC shows significant similarity, with minor differences. The highest incidences are observed in the countries mentioned with inclusion of Russia, the lowest in central and some parts of southern Africa, Northwestern Europe, and North America [7]. The variation in age-standardized incidence rates per 100,000 person-years is marked. It ranges in men from approximately 1 in Botswana and Mozambique to more than 40 in parts of Central Asia such as Mongolia. In women, this range reaches from 0.5 in Botswana and Mozambique to 20 in Guatemala. The latter together with Peru is one of the few countries where the incidence of noncardia cancer is slightly higher in women than in men. Globally, approximately 631,000 GC cases diagnosed in 2012 were male and 320,000 were female. The global male-female ratio is most marked for cardia cancer ranging from 1.5 in Southern Africa to 4 in North America with an average of 3 [7]. For noncardia cancer, the global male-female ratio was approximately 2 with the highest difference in Southeast Asia.

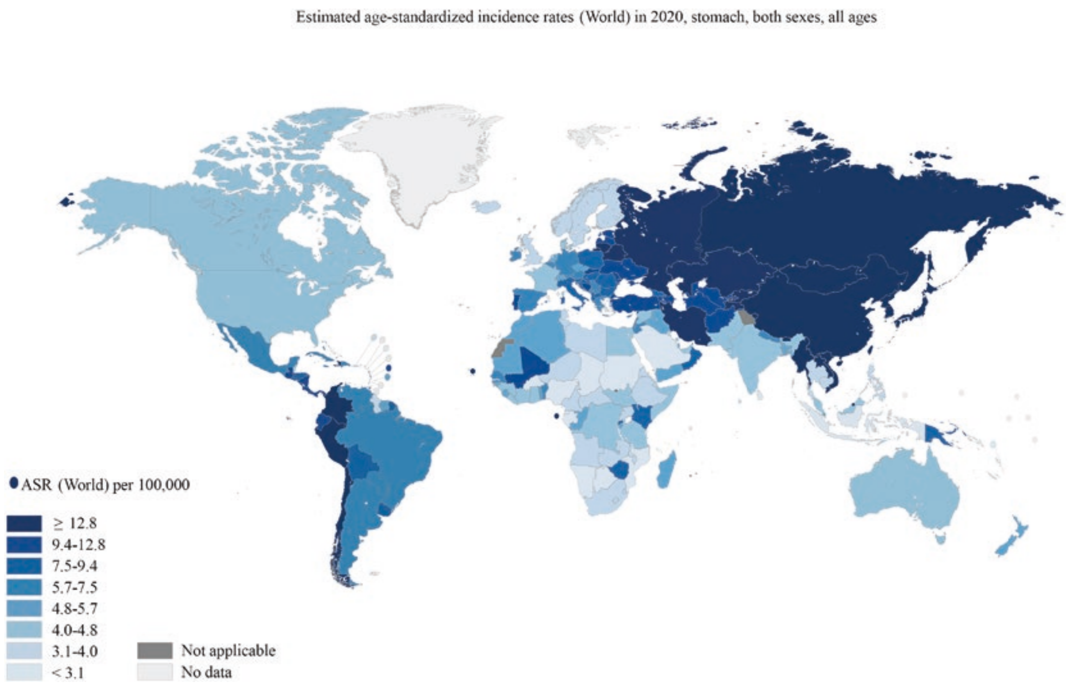

Fig. 22.1 Age-standardized incidence rates of gastric cancer (Adapted from http://globocan.iarc.fr/Default.aspx)

22.2.2 Mortality

The developments in the incidence of GC are closely matched by geographic differences and changes over time in mortality due to the disease [6] (Fig. 22.2). Between 1990 and 2020, the global age-standardized death rate due to GC declined by 55% from 21.7 to 9.8 per 100,000 population [3]. Although this decline was more marked than for any other gastrointestinal (GI) malignancy, the age-standardized death rate for GC remained, after liver and colorectal cancer the third highest of any of GI cancer [6]. Over the same time period, the number of people dying of GC actually remained stable around 770,000 per year [3, 6]. This was due to the increase in size and life expectancy of the world population, in particular, in areas with a high GC incidence. The comparison between annual numbers of newly diagnosed patients (see below) and those dying of the disease shows that even today the global disease fatality rate may amount to above 75%. This fatality rate is significantly lower in countries with population screening programs aiming for early detection of GC [11]. In Japan, the 5-year survival rate is now approximately 69% [12], while even better results are achieved in high-volume centers in Korea [13]. In Western countries, the majority of cases are detected at a more advanced stage. Survival rates for noncardia cancer reach 38%, while survival rates for cardia cancer remain as low as 20% [14], with multimodality treatment contributing to this effect [15]. Observations in the Surveillance Epidemiology and End Results (SEER) database suggest that the difference in GC survival may also be related to host and disease factors, since patients of Asian descent had a 12% better survival than Caucasian patients [16]. This effect persisted after correction for tumor grade and number of lymph nodes investigated. Depending on stage, Asian patients had a 13–72 months longer median survival than corresponding Caucasian patients.

22.2.3 Incidence and Mortality in Lynch Syndrome Carriers

The previous observations were also reflected in the patterns of GC among carriers of a hereditary can-

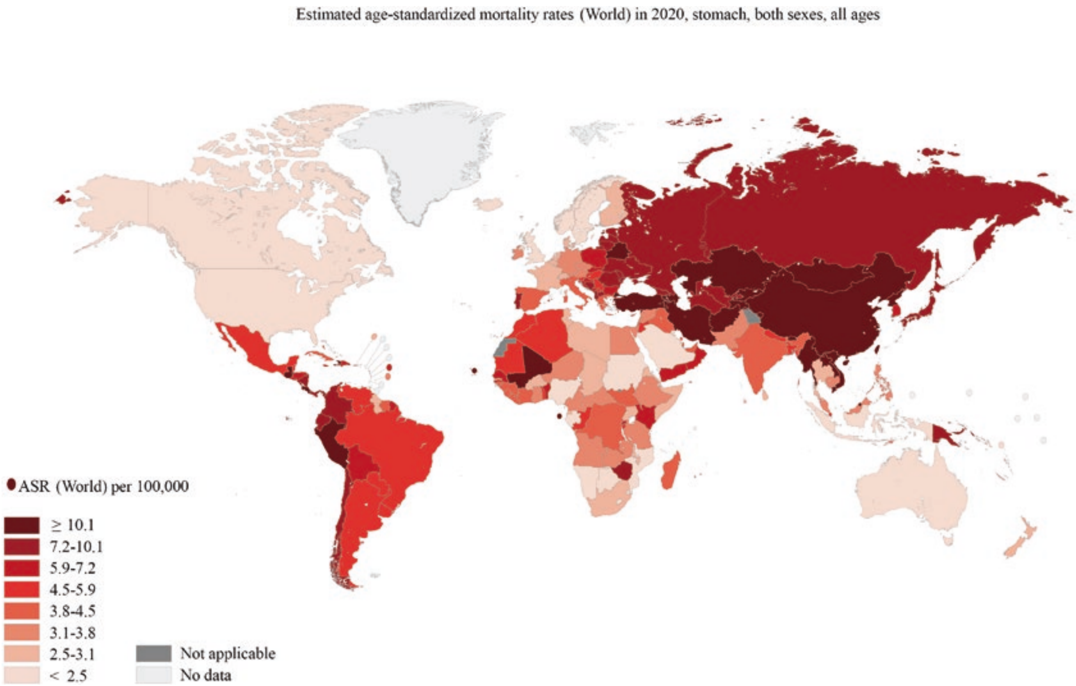

Fig. 22.2 Age-standardized mortality rates of gastric cancer (Adapted from http://globocan.iarc.fr/Default.aspx)

cer syndrome. For instance, in the first description of Lynch syndrome in 1913 in the United States, GC was the predominant cancer affecting family members [17]. This usually already occurred around the age of 40 years. This is thought to have resulted from early exposure to *H. pylori*, most likely in combination with a diet rich in salt and nitrosamines. With changes in diet and a decrease in exposure to *H. pylori*, the predominant cancer in Lynch carriers became colorectal cancer, usually at an age above 40 years. In a nationwide cohort of Lynch carriers in the Netherlands, we observed a 50% decline in GC incidence between 1970 and 2000 [18].

22.3 Risk Factors

22.3.1 *Helicobacter pylori*

The most important risk factor for noncardia GC is *H. pylori* gastritis. The International Agency for Research on Cancer (IARC) thus classified *H. pylori* as a class I carcinogen [19]. Based on nested case-control in longitudinal cohorts [20] as well as retrospective case-control studies, *H. pylori* gastritis has been estimated to be associated with a 17-fold increased risk for development of noncardia GC [21]. Based on this relative risk and the high global prevalence of *H. pylori*, 89% of all noncardia GCs are thought to be attributable to *H. pylori* colonization and the resulting gastritis in all *H. pylori*-positive subjects [22]. This risk depends on the severity of gastritis and is thus higher in those who have more severe chronic active mucosal inflammation as a result of colonization with a more pathogenic *H. pylori* strain [21, 23, 24]. Likewise, GC in *H. pylori*-positives occurs also more frequently in hosts with a more pro-inflammatory genotype [25, 26]. These observations form the basis for the recommendation to consider *H. pylori* eradication either as a population approach in regions with high GC incidence [27, 28] or as targeted approach, for instance, in subjects with a positive family history for GC [29, 30]. When applied prior to the stage of development of intestinal metaplasia, this intervention can strongly reduce the risk of cancer development [31], but it appears to have little preventive effect in those with intestinal metaplasia [31]. In those subjects, cancer can occur even more than a decade after *H. pylori* eradication [32] (see also Chap. 56).

22.3.2 Other Risk Factors

There are a range of other factors that either increase or decrease the risk of GC, in particular factors related to diet [33]. A high intake of salt is associated with an estimated up to threefold increased risk of GC [34]. In a Japanese study that followed 2476 subjects for 14 years, those in the highest quartile of salt intake had a 2.98-fold increased risk of GC compared with those in the lowest quartile [35]. This relative risk remained after correction for confounders such as age, sex, and presence of atrophic gastritis. In a retrospective study from Portugal comparing salt intake in 422 patients with GC versus 649 community controls, the highest tertile of salt intake was associated with a 2.6-fold increased risk for GC [36]. Very similar data were observed elsewhere, such as in China and Latin America [37, 38].

A diet that is short on fresh fruits and vegetables also increases the risk of GC. In a retrospective case-control study from Portugal, the highest tertile of fruit intake in the population was associated with an odds ratio (OR) of 0.47 and 0.53 for, respectively, cardia and noncardia GC [39]. For the highest tertile of vegetable intake, OR were 0.59 and 0.60, these results were in line with a meta-analysis of literature findings [39]. A meta-analysis of a range of studies from South America reported that high intake of fruits and vegetables was associated with 32 and 42% decrease, respectively, in risk of GC, without specification of tumor subsite [38]. The same meta-analysis also reported a 2.3-fold increased risk for GC in those with high intake of chili peppers, but also noted that the data were limited. An increase of risk for GC has also been reported with high intake of processed and red meat, with relative risks ranging from 1.5 to 1.6 in different studies and a meta-analysis from South America [38], while another meta-analysis reported a 15–38% increased risk with every 30 g/day increase in meat consumption [40]. A large European population study following 521,000 men and women for a median 6.5 years concluded that the increased risk with high meat intake was most pronounced in *H. pylori*-positives, with an OR of 5.3 with every 100 g/day increase in meat intake [41].

The risk of GC is increased in smokers. In a European prospective cohort, smoking increased the risk of GC with approximately 80% after adjusting for other factors such as intake of fruit and vegetables [42]. Other studies confirm this correlation [38, 43, 44]. A US population microsimulation model showed that reduction in smoking significantly contributed to the decline in the incidence of noncardia GC and can further do so with in the coming period on top of changes in *H. pylori* prevalence [45].

Other factors that have been associated with GC risk, but less consistent, include alcohol and green tea consumption. In a systematic review of six cohort studies on green tea intake, a limited protective effect was noted on noncardia GC but only in women who drank at least five cups of tea per day [46]. There is no convincing evidence for a modulation of GC risk by coffee consumption [33, 47]. The evidence for a link between both cardia and noncardia GC has not been consistently reported, with a recent meta-analysis concluding that the relative risks were not significantly increased [48].

Together, the abovementioned risk factors have been adequately used for implementation in a prediction model for GC in Korea as a high-incidence country [49]. In Western Europe, as a low-incidence region, it has been estimated that 19% of all GCs and even 62% of all cardia cancers could be prevented by universal adoption of a healthy lifestyle including use of a healthy diet and refraining from smoking and alcohol consumption [50].

22.4 Future Trends

Continuation of the trends of recent decades will likely imply a significant decrease in the standardized incidence of and mortality due to GC. The rapidly expanding and aging world population is nevertheless likely to lead to a further increase in the annual number of newly diagnosed patients. The largest changes are likely to occur in current high-incidence countries, in particular, with changing epidemiology of *H. pylori*. This effect may in some areas such as Japan and Korea be augmented by GC screening programs [51, 52], and further among others in Japan, and parts of China, and Taiwan by population screening programs aiming to screen and treat for *H. pylori* and/or atrophic gastritis [27, 31, 53].

A similar effect may occur in Western countries, even in those that already have low incidences of GC. It has, for instance, been estimated that the decrease would be 66% for mortality and 50% for incidence within Europe when comparing 2030 with 2005 [54]. This estimate is supported by the observation of a marked decrease in the prevalence of atrophic gastritis and intestinal metaplasia in the general population [55]. Since these lesions are predominant risk factors for later development of GC, their prevalence is a looking glass for the incidence of GC in a population in the following two decades [56]. However, despite the decrease, atrophic gastritis and intestinal metaplasia remain common conditions in those above the age of 50 years even in Western Europe [55, 57].

Some effects may however interfere with this trend. These include the observation that cardia cancer is becoming predominant in Western countries [7]. The incidence of this tumor shows much less of a change over the past decades. For instance, in the United States, the incidence of cardia cancer actually tended to increase between 1976 and 2007 in all racial groups [9]. Over the same time period, the incidence of noncardia GC stabilized or even slightly increased in men and women below the age of 50 years [58]. A similar stabilization of noncardia GC has recently been observed in the Netherlands [59]. Furthermore, there are some observations that the decreasing prevalence of *H. pylori* in subsequent birth cohorts may have slowed or stopped in a Western country such as the Netherlands for reasons which are not yet fully understood, but may include a marked increase in the use daycare at very young age [60, 61].

22.5 Conclusions

GC has for long been the most common human malignancy. Despite a marked decline in incidence during the past decades, it still is the fifth most common cancer accounting for close to 7% of all human cancers. The disease is more common in men than in women, and more often affects the body and antrum of the stomach than the cardia. Despite the decrease in incidence, GC remains the most common cause of GI cancer-related death, with more than 800,000 fatalities annually. There are large regional and racial differences in the incidence of GC. These differences are related to the prevalence of *H. pylori*, diet, and other risk factors. The mortality of GC closely matches the regional differences in incidence. Globally, the majority of patients affected by GC still die as a result of the disease. The high incidences and fatality rates are remarkable in view of the fact that GC is largely a preventable disease and can also be detected and treated with excellent outcome at a precursor or early cancer stage. The age-standardized incidence and mortality rates of GC are expected to further decrease due to improvement in socioeconomic conditions and decreasing prevalence of *H. pylori*. Population screening and intervention, as well as general health measures such as antismoking campaigns, can accelerate the changing epidemiology of GC. In the absence of such measures, GC will for long remain a very common and lethal disease.

References

1. Boas I. Diagnostik und Therapie der Magenkrankheiten. Leipzig: Georg Thieme; 1925.
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, et al. Cancer statistics, 2008. CA Cancer J Clin. 2008;58:71–96.
3. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;385:117–71.
4. Fock KM. Review article: the epidemiology and prevention of gastric cancer. Aliment Pharmacol Ther. 2014;40:250–60.
5. Tanaka M, Ma E, Tanaka H, Ioka A, Nakahara T, Takahashi H. Trends of stomach cancer mortality in eastern Asia in 1950-2004: comparative study of Japan, Hong Kong and Singapore using age, period and cohort analysis. Int J Cancer. 2012;130:930–6.
6. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:191–280.
7. Thrift AP, Wenker TN, El-Serag HB. Global burden of gastric cancer: epidemiological trends, risk factors, screening and prevention. Nat Rev Clin Oncol. 2023;20:338–49.

8. Arnold M, Moore SP, Hassler S, Ellison-Loschmann L, Forman D, Bray F. The burden of stomach cancer in indigenous populations: a systematic review and global assessment. Gut. 2014;63:64–71.
9. Camargo MC, Anderson WF, King JB, Correa P, Thomas CC, Rosenberg PS, et al. Divergent trends for gastric cancer incidence by anatomical subsite in US adults. Gut. 2011;60:1644–9.
10. Lim KG. Malays in peninsular Malaysia may have the lowest incidence of stomach cancer in the world. Med J Malaysia. 2009;64:91–2.
11. Kimman M, Norman R, Jan S, Kingston D, Woodward M. The burden of cancer in member countries of the Association of Southeast Asian Nations (ASEAN). Asian Pac J Cancer Prev. 2012;13:411–20.
12. Isobe Y, Nashimoto A, Akazawa K, Oda I, Hayashi K, Miyashiro I, et al. Gastric cancer treatment in Japan: 2008 annual report of the JGCA nationwide registry. Gastric Cancer. 2011;14:301–16.
13. Shen ZL, Song KY, Ye YJ, Xie QW, Liang B, Jiang K, et al. Significant differences in the clinicopathological characteristics and survival of gastric cancer patients from two cancer centers in China and Korea. J Gastric Cancer. 2015;15:19–28.
14. Libanio D, Rodrigues JR, Bento MJ, Ebigbo A, Messman H, Verhoeven RH, et al. Gastric cancer incidence and mortality trends 2007-2016 in three European countries. Endoscopy. 2022;54:644–52.
15. Seyedin S, Wang PC, Zhang Q, Lee P. Benefit of adjuvant chemoradiotherapy for gastric adenocarcinoma: a SEER population analysis. Gastrointest Cancer Res. 2014;7:82–90.
16. Wang J, Sun Y, Bertagnolli MM. Comparison of gastric cancer survival between Caucasian and Asian patients treated in the United States: results from the surveillance epidemiology and end results (SEER) database. Ann Surg Oncol. 2015;22:2965–71.
17. Warthin A. Heredity with reference to carcinoma: as shown by the study of the cases examined in the pathological laboratory of the University of Michigan. Arch Intern Med. 1913;12:546–55.
18. Capelle LG, Van Grieken NC, Lingsma HF, Steyerberg EW, Klokman WJ, Bruno MJ, et al. Risk and epidemiological time trends of gastric cancer in lynch syndrome carriers in The Netherlands. Gastroenterology. 2010;138:487–92.
19. Schistosomes, liver flukes and *Helicobacter pylori*. IARC Working Group on the evaluation of carcinogenic risks to humans. Lyon, 7–14 June 1994. IARC Monogr Eval Carcinog Risks Hum 1994;61:1–241.
20. Helicobacter and Cancer Collaborative Group. Gastric cancer and *Helicobacter pylori*: a combined analysis of 12 case control studies nested within prospective cohorts. Gut. 2001;49:347–53.
21. Simán JH, Engstrand L, Berglund G, Forsgren A, Florén CH. *Helicobacter pylori* and CagA seropositivity and its association with gastric and oesophageal carcinoma. Scand J Gastroenterol. 2007;42: 933–40.
22. Plummer M, Franceschi S, Vignat J, Forman D, de Martel C. Global burden of gastric cancer attributable to *Helicobacter pylori*. Int J Cancer. 2015;136: 487–90.
23. Kuipers EJ, Pérez-Pérez GI, Meuwissen SG, Blaser MJ. *Helicobacter pylori* and atrophic gastritis: importance of the cagA status. J Natl Cancer Inst. 1995;87:1777–80.
24. Basso D, Zambon CF, Letley DP, Stranges A, Marchet A, Rhead JL, et al. Clinical relevance of *Helicobacter pylori* cagA and vacA gene polymorphisms. Gastroenterology. 2008;135:91–9.
25. Persson C, Canedo P, Machado JC, El-Omar EM, Forman D. Polymorphisms in inflammatory response genes and their association with gastric cancer: a HuGE systematic review and meta-analyses. Am J Epidemiol. 2011;173:259–70.
26. Hunt RH, Camilleri M, Crowe SE, El-Omar EM, Fox JG, Kuipers EJ, et al. The stomach in health and disease. Gut. 2015;64:1650–68.
27. Lee YC, Chen TH, Chiu HM, Shun CT, Chiang H, Liu TY, et al. The benefit of mass eradication of *Helicobacter pylori* infection: a community-based study of gastric cancer prevention. Gut. 2013;62:676–82.
28. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, et al. Kyoto global consensus report on management of *Helicobacter pylori* gastritis. Gut. 2015;64:1353–67.
29. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of *Helicobacter pylori* infection—the Maastricht IV/ Florence Consensus Report. Gut. 2012;61:646–64.
30. Dinis-Ribeiro M, Areia M, de Vries AC, Marcos-Pinto R, Monteiro-Soares M, O'Connor A, et al. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa. Endoscopy. 2012;44: 74–94.
31. Wong BC, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, et al. *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. JAMA. 2004;291:187–94.
32. de Vries AC, Kuipers EJ, Rauws EA. *Helicobacter pylori* eradication and gastric cancer: when is the horse out of the barn? Am J Gastroenterol. 2009;104:1342–5.
33. Fang X, Wei J, He X, An P, Wang H, Jiang L, et al. Landscape of dietary factors associated with risk of gastric cancer: a systematic review and dose-response meta-analysis of prospective cohort studies. Eur J Cancer. 2015;51:2820–32.
34. Wang XQ, Terry PD, Yan H. Review of salt consumption and stomach cancer risk: epidemiological and biological evidence. World J Gastroenterol. 2009;15:2204–13.
35. Shikata K, Kiyohara Y, Kubo M, Yonemoto K, Ninomiya T, Shirota T, et al. A prospective study of dietary salt intake and gastric cancer incidence in a

defined Japanese population: the Hisayama study. Int J Cancer. 2006;119:196–201.

36. Peleteiro B, Lopes C, Figueiredo C, Lunet N. Salt intake and gastric cancer risk according to *Helicobacter pylori* infection, smoking, tumour site and histological type. Br J Cancer. 2011;104:198–207.
37. Zhong C, Li KN, Bi JW, Wang BC. Sodium intake, salt taste and gastric cancer risk according to *Helicobacter pylori* infection, smoking, histological type and tumor site in China. Asian Pac J Cancer Prev. 2012;13:2481–4.
38. Bonequi P, Meneses-González F, Correa P, Rabkin CS, Camargo MC. Risk factors for gastric cancer in Latin America: a meta-analysis. Cancer Causes Control. 2013;24:217–31.
39. Lunet N, Valbuena C, Vieira AL, Lopes C, Lopes C, David L, et al. Fruit and vegetable consumption and gastric cancer by location and histological type: case-control and meta-analysis. Eur J Cancer Prev. 2007;16:312–27.
40. Larsson SC, Orsini N, Wolk A. Processed meat consumption and stomach cancer risk: a meta-analysis. J Natl Cancer Inst. 2006;98:1078–87.
41. González CA, Jakszyn P, Pera G, Agudo A, Bingham S, Palli D, et al. Meat intake and risk of stomach and esophageal adenocarcinoma within the European Prospective Investigation Into Cancer and Nutrition (EPIC). J Natl Cancer Inst. 2006;98:345–54.
42. González CA, Pera G, Agudo A, Palli D, Krogh V, Vineis P, et al. Smoking and the risk of gastric cancer in the European Prospective Investigation Into Cancer and Nutrition (EPIC). Int J Cancer. 2003;107:629–34.
43. Steevens J, Schouten LJ, Goldbohm RA, van den Brandt PA. Alcohol consumption, cigarette smoking and risk of subtypes of oesophageal and gastric cancer: a prospective cohort study. Gut. 2010;59:39–48.
44. Praud D, Rota M, Pelucchi C, Bertuccio P, Rosso T, Galeone C, et al. Cigarette smoking and gastric cancer in the stomach cancer pooling (StoP) project. Eur J Cancer Prev. 2018;27:124–33.
45. Yeh JM, Hur C, Schrag D, Kuntz KM, Ezzati M, Stout N, et al. Contribution of *H. pylori* and smoking trends to US incidence of intestinal-type noncardia gastric adenocarcinoma: a microsimulation model. PLoS Med. 2013;10:e1001451.
46. Inoue M, Sasazuki S, Wakai K, Suzuki T, Matsuo K, Shimazu T, et al. Green tea consumption and gastric cancer in Japanese: a pooled analysis of six cohort studies. Gut. 2009;58:1323–32.
47. Liu H, Hua Y, Zheng X, Shen Z, Luo H, Tao X, et al. Effect of coffee consumption on the risk of gastric cancer: a systematic review and meta-analysis of prospective cohort studies. PLoS One. 2015;10:e0128501.
48. Tramacere I, Negri E, Pelucchi C, Bagnardi V, Rota M, Scotti L, et al. A meta-analysis on alcohol drinking and gastric cancer risk. Ann Oncol. 2012;23:28–36.
49. Eom BW, Joo J, Kim S, Shin A, Yang HR, Park J, et al. Prediction model for gastric cancer incidence in Korean population. PLoS One. 2015;10:e0132613.
50. Buckland G, Travier N, Huerta JM, Bueno-de-Mesquita HB, Siersema PD, Skeie G, et al. Healthy lifestyle index and risk of gastric adenocarcinoma in the EPIC cohort study. Int J Cancer. 2015;137:598–606.
51. Sugano K. Screening of gastric cancer in Asia. Best Pract Res Clin Gastroenterol. 2015;29:895–905.
52. Choi KS, Jun JK, Suh M, Park B, Noh DK, Song SH, et al. Effect of endoscopy screening on stage at gastric cancer diagnosis: results of the national cancer screening programme in Korea. Br J Cancer. 2015;112:608–12.
53. Yoshida T, Kato J, Inoue I, Yoshimura N, Deguchi H, Mukoubayashi C, et al. Cancer development based on chronic active gastritis and resulting gastric atrophy as assessed by serum levels of pepsinogen and *Helicobacter pylori* antibody titer. Int J Cancer. 2014;134:1445–57.
54. Amiri M, Janssen F, Kunst AE. The decline in stomach cancer mortality: exploration of future trends in seven European countries. Eur J Epidemiol. 2011;26:23–8.
55. de Vries AC, Meijer GA, Looman CW, Casparie MK, Hansen BE, van Grieken NC, et al. Epidemiological trends of pre-malignant gastric lesions: a long-term nationwide study in The Netherlands. Gut. 2007;56:1665–70.
56. de Vries AC, van Grieken NC, Looman CW, Casparie MK, de Vries E, Meijer GA, et al. Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in The Netherlands. Gastroenterology. 2008;134:945–52.
57. den Hoed CM, van Eijck BC, Capelle LG, van Dekken H, Biermann K, Siersema PD, et al. The prevalence of premalignant gastric lesions in asymptomatic patients: predicting the future incidence of gastric cancer. Eur J Cancer. 2011;47:1211–8.
58. Anderson WF, Rabkin CS, Turner N, Fraumeni JF, Rosenberg PS, Camargo MC. The changing face of noncardia gastric cancer among US non-Hispanic whites. J Natl Cancer Inst. 2018;110:608–15.
59. Holster IL, Aarts MJ, Tjwa ET, Lemmens VE, Kuipers EJ. Trend breaks in incidence of non-cardia gastric cancer in The Netherlands. Cancer Epidemiol. 2014;38:9–15.
60. Roosendaal R, Kuipers EJ, Buitenwerf J, van Uffelen C, Meuwissen SG, van Kamp GJ, et al. *Helicobacter pylori* and the birth cohort effect: evidence of a continuous decrease of infection rates in childhood. Am J Gastroenterol. 1997;92:1480–2.
61. den Hoed CM, Vila AJ, Holster IL, Perez-Perez GI, Blaser MJ, de Jongste JC, et al. *Helicobacter pylori* and the birth cohort effect: evidence for stabilized colonization rates in childhood. Helicobacter. 2011;16:405–9.

23

Gastric Cancer: Genetic Alternations Induced by *H. pylori* Infection: The Role of Activation-Induced Cytidine Deaminase

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Abstract

Oxidative stress and aberrant activation-induced cytidine deaminase (AID) expression induced by chronic inflammation play an important role in inflammation-associated carcinogenesis. While AID plays a physiological role in B lymphocytes to induce somatic hypermutation and class switch recombination of immunoglobulin gene, it appears to promote mutagenesis in other cells than B lymphocytes. The impact of AID on carcinogenesis has been reported in numerous studies on colon, liver, lung, and gastric cancers, as well as hematologic malignancies. Chronic active gastritis by *Helicobacter pylori* (*H. pylori*) infection leads to aberrant AID expression, followed by mutations of tumor-related genes such as *p53* and *CDKN2B-CDKN2A*, which suggests one possible mechanism how *H. pylori* infection induces gastric carcinogenesis.

Keywords

Helicobacter pylori · Mutation · Reactive oxygen species · Activation-induced cytidine deaminase

23.1 Introduction

Helicobacter pylori (*H. pylori*)-associated chronic gastritis induces altered cellular signaling, imbalanced proliferation, and apoptosis of gastric epithelial cells via genetic mutations and/or epigenetic changes (aberrant DNA methylation and microRNA dysregulation), both of which modulate gene expression and genomic instability, driving the progression from precancerous lesions to cancer. As a matter of fact, *H. pylori*-induced gastric carcinogenesis is a complex process and could not be easily explained by a single mechanism. In this chapter, how chronic inflammation can induce mutagenesis and cancer will be highlighted. In addition, how *H. pylori* manipulates the host nucleotide-editing enzyme activation-induced cytidine deaminase (AID) and promotes genomic mutations in the gastric epithelium will be discussed.

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23.2 Genetic Alternations in Gastric Carcinogenesis

Carcinogenesis can be defined as a stepwise accumulation of genetic and epigenetic alterations that drives the progressive transformation of normal cells into malignant derivatives [1]. Mutagenesis in gastric carcinogenesis includes microsatellite instability (MSI) resulting from altered DNA mismatch repair, point mutations, and genomic instability including loss of heterozygosity (LOH), gene amplifications, rearrangements, insertion and deletion mutations, chromosomal losses and duplications, and DNA copy number variation.

The spectrum of mutations in gastric cancer (GC) has been explored by massive parallel sequencing approaches. A recent study performed whole exome sequencing in GC, and it identified frequently mutated genes and involved molecular pathways [2]. It was found that chromatin modification and cell junction organization were the pathways with the most significant enrichment of mutated genes [3]. That is, mutations were found in members of the SWI-SNF complex (*ARID1A*, *PBRM1*, and *SMARCC1*), ISWI complex (*SMARCA1*), and NuRD complex (*CHD3*, *CHD4*, and *MBD2*), and other genes encoding histone-modifying proteins (*SIRT1* and *SETD2*), in 59% of GC samples. In addition, 59% of GC samples had mutations in genes involved in cell adhesion, including *CDH1* and other cadherin family members, which shows the infiltrative nature of GC and the importance of the loss of cell-to-cell adhesion in gastric carcinogenesis. Genes involved in cell cycle regulation such as *TP53*, *PTEN*, and *TTK* were mutated in 77% of GCs. Other signaling pathways frequently mutated in GCs included the Wnt-BMP-TGFβ, axon guidance, MAPK, DNA replication, focal adhesion, ERBB, ATR-BRCA, and Rb pathways. Mutations or protein deficiency of *ARID1A* was frequent in 83% of GCs with MSI and 73% with Epstein-Barr virus (EBV) infection, but it was infrequent in 11% that were microsatellite stable (MSS) without EBV infection. Thus, the frequency of *ARID1A* mutation was different between molecular subtypes of GC, and it was negatively associated with that of *TP53* mutation [2]. Another study also performing exome sequencing has reported that cell-to-cell adhesion was the pathway most enriched for mutations [3]. *TP53* was mutated in 66.7%, and *PIK3CA* and *ARIDI1A* were mutated in 20% of GCs. Frequent mutations in chromatin remodeling genes (*ARIDI1A*, *MLL3*, and *MLL*) were found in 47% of GCs. *ARIDI1A* mutations were associated with *PIK3CA* mutations and MSI [3].

23.3 Chronic Inflammation and Genetic Alternations

As mentioned above, genomic mutations accumulate during the steps of gastric carcinogenesis in cells representing intestinal metaplasia, dysplasia/adenoma, and adenocarcinoma and probably in epithelial progenitors and stem cells. In the multistep gastric carcinogenesis, genetic alternation of tumor suppressor gene *TP53* and *APC* is most frequently observed [4]. Mutation of *TP53* is common in noncancerous gastric mucosae with chronic inflammation or precancerous lesions [5]. *APC* mutations, including stop-codon and frameshift mutations, were reported in 40% of gastric flat adenomas [6].

Among digestive organ cancers, GCs are among the class of cancers that arise in association with chronic inflammation (chronic active gastritis), like other gastrointestinal tract cancers such as esophageal adenocarcinoma associated with reflux esophagitis and Barrett's esophagus and colorectal cancers in patients with inflammatory bowel disease [7]. To date, there are two possible intrinsic mutagens responsible for genetic aberrations in the inflammatory condition: (1) free radicals and (2) intrinsic DNA mutator enzymes [7].

23.3.1 Free Radicals

Chronic inflammation induces reactive oxygen species (ROS) and reactive nitrogen species, and oxidative stress caused by ROS increases DNA damage of epithelial cells. ROS generates various modified bases of oxidatively altered purines or pyrimidines, which induce the putative DNA damages such as single- or double-stranded DNA breaks, DNA intrastrand adducts, and DNA protein cross-links [8, 9]. In addition, ROS can also induce mismatch repair function and mutation of microsatellite sequences, and the genetic instability is closely related to not only cancers but precancerous lesions. In this regard, ROS is a putative mediator that links excessive activity of oncogene products and DNA damage [7].

23.3.2 Activation-Induced Cytidine Deaminase

However, oxidative stress induced by ROS cannot fully explain the mutagenesis observed in many human cancers, particularly in inflammation-associated cancers [7]. Recently, several human enzymes that are capable of inducing nucleotide alterations have been identified, providing a new concept for understanding the mechanisms of mutagenesis. Among them, AID is a well-defined molecule involved in DNA mutations in the human genome [10]. Through its enzymatic activity, AID can deaminate cytidine (C) on target DNA to uracil (U), leading to the change of a DNA C:G pair into a U:G mismatch. When DNA replication starts before being recognized by the repair system, a U:G mismatch gives rise to C/G to T/A transition [11].

Under physiological conditions, AID contributes to promoting antibody gene diversification in germinal center B cells by inducing somatic hypermutation and class switch recombination of immunoglobulin gene [12]; it leads to the maintenance of the immune system diversity. While AID plays a favorable role in the immune system, aberrant AID activity might affect nonimmunoglobulin genes, including tumor-related genes in nonlymphoid cells.

The role of AID in tumorigenesis is the induction of genetic instability, which was first suggested in hematopoietic malignancies [13]. A number of studies have shown that increased AID expression was associated with unfavorable mutations and chromosomal translocations in various malignancies of the B lymphocytic lineage [14, 15]. AID has been shown to be responsible for the chromosomal breaks in c-MYC, leading to a c-MYC/IgH translocation in B-cell lymphoma [16]. Moreover, AID induces breakpoint cluster region-Abelson murine leukemia viral oncogene homolog 1 mutations, resulting in the imatinib resistance in chronic myeloid leukemia cells [17]. Because the target of AID-mediated genotoxic effects was not only immunoglobulin genes but numerous other genes, aberrant AID expression induced genetic alterations in various tumor-related genes, leading to the transformation of the hematopoietic cells. Recently, there have been several reports on the impact of AID expression in gastric and colorectal cancers and HCV-related hepatocellular carcinoma. Especially, AID transgenic mice developed neoplasia in epithelial tissues including the lung, liver, and stomach, related to *Trp53* mutations. This result indicates that aberrant AID expression in epithelial cells can promote carcinogenesis by genetic instability [18]. Interestingly, the frequently mutated tumor-related genes induced by aberrant AID expression differ among different cancers. For example, while the mutations of *c-Myc* were usually detected in lung cancer, *K-ras* mutations were frequently observed in GC [19], which indicates the diversity of organ-specific oncogenic pathways in various epithelial tissues.

Nuclear factor (NF)-κB can be activated in response to various inflammatory stimulations, which is closely related to the multiple processes of initiation and progression in carcinogenesis. NF-κB is known as a major transcription factor for AID [20]. Increased expression of AID induced by tumor necrosis factor (TNF)-α stimuli has been reported in *H. pylori*-related gastric carcinogenesis [21], as well as colitis-associated colorectal carcinogenesis [22]. In human colon cancer cell lines, AID expression could be induced in STAT6-dependent manner.

23.4 Genetic Alternations in *H. pylori*-Associated Gastric Carcinogenesis

In *H. pylori*-associated gastric carcinogenesis, DNA damage of epithelial cells is attributed to the oxidative stress caused by ROS and reactive nitrogen species generated by inflammatory cells as well as by gastric epithelial cells activated by *H. pylori*. In addition, epithelial expression of AID in *H. pylori*-associated gastritis may induce C/G to T/A transitions by its cytidine deaminase activity [21], which is regarded as one of the mechanisms in gastric carcinogenesis.

While endogenous AID expression is not detected in normal epithelial cells under physiological conditions, it has been observed in the *H. pylori*-infected gastric epithelium. As mentioned above, NF-κB is an important transcription factor for AID expression. According to the previous studies, AID expression in human GC cell lines could be induced by NF-κB activation stimulated by TNF-α, but was not observed if there was no TNF-α stimulus. That is, *H. pylori* infection promotes aberrant AID expression in gastric mucosae via increased production of inflammatory cytokines such as TNF-α and interleukin (IL)-1β. In addition, pathogenic *H. pylori* strain per se is able to induce AID expression, too; there is evidence that cytotoxin-associated gene (*cag*) pathogenicity island (PAI)-positive strains could activate the NF-κB pathway via type IV secretion system; *cag* PAI-positive *H. pylori*-derived virulence factors (peptidoglycans) injected into the gastric epithelial cells induce the NF-κB activation [23] (Fig. 23.1). AID in the gastric epithelium in the setting of *H. pylori* infection can lead to increased U:G mismatches

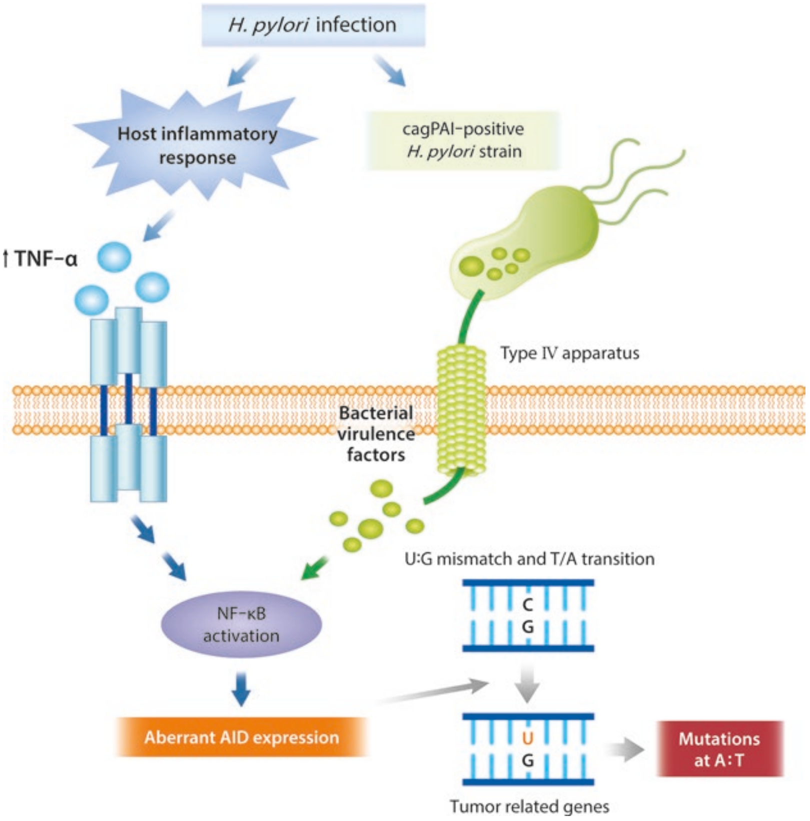

Fig. 23.1 The role of *Helicobacter pylori* infection in gastric carcinogenesis in view of aberrant activation-induced cytidine deaminase (AID) expression. AID acts as a cytidine deaminase that is capable of inducing nucleotide alterations in human DNA sequences. *H. pylori* infection can induce AID expression in gastric epithelial cells via two distinct pathways. First, *cag* PAI-positive *H. pylori* strains per se possess type IV machinery and can inject bacterial virulence factors into gastric epithelial cells, leading to the activation of the host transcriptional factor NF-κB. Second, the host inflammatory response triggered by *H. pylori* infection can also activate NF-κB in the gastric epithelium. As a result, AID is transcriptionally upregulated in gastric epithelial cells and can contribute to the mutagenesis of tumor-related genes (Modified from Marusawa and Chiba [23])

further contributing to the number of unrepaired mismatches and increased T/A transitions. Gene targets of AID-enhanced mutagenesis in gastric cells include *TP53* and *CDKN2B-CDKN2A* (encoding p16, p15, and p14 suppressor proteins) and caused submicroscopic deletions with chromosome copy number losses involving the *CDKN2B-CDKN2A* locus [21, 24]. Interestingly, one recent study showed that *H. pylori* eradication attenuated aberrant AID expression in the gastric epithelium, which suggests one possible mechanism that anti-*H. pylori* treatment can reduce the risk of GC [25].

In contrast to the significant role in gastric adenocarcinoma, AID appears to play a limited role in *H. pylori*-associated mucosa-associated lymphoid tissue (MALT) lymphoma. According to the previous reports on nongastric MALT lymphomas, aberrant AID expression was confined to the reactive areas; AID was rarely expressed in neoplastic marginal zone B cells [26, 27]. In addition, it remains unknown whether *H. pylori* infection enhances aberrant AID expression in gastric B lymphocytes [23]. Therefore, further study is necessary in this issue.

23.5 Conclusions

Chronic inflammation plays important roles in gastric carcinogenesis. During chronic inflammation, intrinsic mediators of inflammatory responses, including pro-inflammatory cytokines and reactive oxygen and nitrogen species, can induce genetic mutations. Recently, the role of AID as a genomic modulator has been highlighted in *H. pylori*-associated GC development.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57–70.
2. Wang K, Kan J, Yuen ST, Shi ST, Chu KM, Law S, et al. Exome sequencing identifies frequent mutation of ARID1A in molecular subtypes of gastric cancer. Nat Genet. 2011;43:1219–23.
3. Zang ZJ, Cutcutache I, Poon SL, Zhang SL, McPherson JR, Tao J, et al. Exome sequencing of gastric adenocarcinoma identifies recurrent somatic mutations in cell adhesion and chromatin remodeling genes. Nat Genet. 2012;44:570–4.
4. Joerger AC, Fersht AR. Structure-function-rescue: the diverse nature of common p53 cancer mutants. Oncogene. 2007;26:2226–42.
5. Shiao YH, Rugge M, Correa P, Lehmann HP, Scheer WD. p53 alteration in gastric precancerous lesions. Am J Pathol. 1994;144:511–7.
6. Nakatsuru S, Yanagisawa A, Furukawa Y, Ichii S, Kato Y, Nakamura Y, et al. Somatic mutations of the APC gene in precancerous lesion of the stomach. Hum Mol Genet. 1993;2:1463–5.
7. Chiba T, Marusawa H, Ushijima T. Inflammation-associated cancer development in digestive organs: mechanisms and roles for genetic and epigenetic modulation. Gastroenterology. 2012;143:550–63.
8. Demple B, Harrison L. Repair of oxidative damage to DNA: enzymology and biology. Annu Rev Biochem. 1994;63:915–48.
9. Federico A, Morgillo F, Tuccillo C, Ciardiello F, Loguercio C. Chronic inflammation and oxidative stress in human carcinogenesis. Int J Cancer. 2007;121:2381–6.
10. Conticello SG. The AID/APOBEC family of nucleic acid mutators. Genome Biol. 2008;9:229.
11. Liu M, Duke JL, Richter DJ, Vinuesa CG, Goodnow CC, Kleinstein SH, et al. Two levels of protection for the B cell genome during somatic hypermutation. Nature. 2008;451:841–5.
12. Honjo T, Kinoshita K, Muramatsu M. Molecular mechanism of class switch recombination: linkage with somatic hypermutation. Annu Rev Immunol. 2002;20:165–96.
13. Marusawa H. Aberrant AID, expression and human cancer development. Int J Biochem Cell Biol. 2008;40:1399–402.
14. Greeve J, Philipsen A, Krause K, Klapper W, Heidorn K, Castle BE, et al. Expression of activation-induced cytidine deaminase in human B-cell non-Hodgkin lymphomas. Blood. 2003;101:3574–80.
15. Pasqualucci L, Guglielmino R, Houldsworth J, Mohr J, Aoufouchi S, Polakiewicz R, et al. Expression of the AID protein in normal and neoplastic B cells. Blood. 2004;104:3318–25.
16. Robbiani DF, Bothmer A, Callen E, Reina-San-Martin B, Dorsett Y, Difilippantonio S, et al. AID is required for the chromosomal breaks in c-myc that lead to c-myc/IgH translocations. Cell. 2008;135:1028–38.
17. Klemm L, Duy C, Iacobucci I, Kuchen S, von Levetzow G, Feldhahn N, et al. The B cell mutator AID promotes B lymphoid blast crisis and drug resistance in chronic myeloid leukemia. Cancer Cell. 2009;16:232–45.
18. Okazaki IM, Hiai H, Kakazu N, Yamada S, Muramatsu M, Kinoshita K, et al. Constitutive expression of AID leads to tumorigenesis. J Exp Med. 2003;197:1173–81.

19. Morisawa T, Marusawa H, Ueda Y, Iwai A, Okazaki IM, Honjo T, et al. Organ-specific profiles of genetic changes in cancers caused by activation-induced cytidine deaminase expression. Int J Cancer. 2008;123:2735–40.
20. Nagaoka H, Tran TH, Kobayashi M, Aida M, Honjo T. Preventing AID, a physiological mutator, from deleterious activation: regulation of the genomic instability that is associated with antibody diversity. Int Immunol. 2010;22:227–35.
21. Matsumoto Y, Marusawa H, Kinoshita K, Endo Y, Kou T, Morisawa T, et al. *Helicobacter pylori* infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. Nat Med. 2007;13:470–6.
22. Endo Y, Marusawa H, Kou T, Nakase H, Fujii S, Fujimori T, et al. Activation-induced cytidine deaminase links between inflammation and the development of colitis-associated colorectal cancers. Gastroenterology. 2008;135:889–98, 898 e881–3.
23. Marusawa H, Chiba T. *Helicobacter pylori*-induced activation-induced cytidine deaminase expression and carcinogenesis. Curr Opin Immunol. 2010;22:442–7.
24. Matsumoto Y, Marusawa H, Kinoshita K, Niwa Y, Sakai Y, Chiba T. Up-regulation of activation-induced cytidine deaminase causes genetic aberrations at the CDKN2b-CDKN2a in gastric cancer. Gastroenterology. 2010;139:1984–94.
25. Nagata N, Akiyama J, Marusawa H, Shimbo T, Liu Y, Igari T, et al. Enhanced expression of activation-induced cytidine deaminase in human gastric mucosa infected by *Helicobacter pylori* and its decrease following eradication. J Gastroenterol. 2014;49:427–35.
26. Bombardieri M, Barone F, Humby F, Kelly S, McGurk M, Morgan P, et al. Activation-induced cytidine deaminase expression in follicular dendritic cell networks and interfollicular large B cells supports functionality of ectopic lymphoid neogenesis in autoimmune sialoadenitis and MALT lymphoma in Sjogren's syndrome. J Immunol. 2007;179:4929–38.
27. Deutsch AJ, Aigelsreiter A, Staber PB, Beham A, Linkesch W, Guelly C, et al. MALT lymphoma and extranodal diffuse large B-cell lymphoma are targeted by aberrant somatic hypermutation. Blood. 2007;109:3500–4.

24

Gastric Cancer: Epigenetic Mechanisms: Aberrant DNA Methylation and Dysregulation of MicroRNA

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Abstract

Like many human cancers, global DNA hypomethylation and promoter CpG island hypermethylation in tumor suppressor or tumor-related genes are frequently observed in gastric cancer (GC), and aberrant DNA methylation occurs in a gene-specific manner during the multistep gastric carcinogenesis. Chronic *Helicobacter pylori* (*H. pylori*) infection induces pro-inflammatory cytokines and reactive oxygen and nitrogen species in the gastric mucosa, which is known to be associated with the accumulation of aberrant DNA methylation. Aberrant DNA methylation caused by *H. pylori*-associated gastritis persists even after the disappearance of *H. pylori*, and epigenetic alterations induced by *H. pylori* correlate with the risk for GC. Numerous microRNAs (miRNAs) are dysregulated during the gastric carcinogenesis, and some of these miRNAs are known to be also dysregulated by *H. pylori* infection. miRNAs dysregulated by *H. pylori* infection play an important role in gastric carcinogenesis by modulating inflammation and immune response of the host, cell-cycle progression, apoptosis and proliferation, and tumor invasion and metastasis. Epigenetic alternations induced by *H. pylori* can be a surrogate marker for predicting GC risk in the future.

Keywords

Helicobacter pylori · Epigenetics · Gastric cancer · Methylation · MicroRNA

24.1 Introduction

Aberrant DNA methylation is a major epigenetic mechanism associated with gene silencing; it is observed in many human cancers [1]. Global DNA hypomethylation and promoter hypermethylation in the specific genes have been associated with genomic instability and inactivation of tumor suppressor genes, respectively. Global hypomethylation mostly occurs in the intergenic region, and it precipitates chromosomal instability which leads to chromosomal mutations such as recombination, translocation, deletion, and rearrangement [2, 3]. However, the promoter CpG island hypermethylation induces carcinogenesis by gene silencing of mostly tumor suppressor genes. These epigenetic changes are known to be replicated with a high fidelity in mammalian cells, mediated by DNA methyltransferases (DNMTs) which add methyl groups to cytosines and in result serve as a long-term memory of cells.

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In gastric cancer (GC), tumor suppressor or tumor-related genes are more frequently inactivated by CpG island hypermethylation than by mutations [4]. To date, promoter hypermethylation of such genes as *p16*, *CDH1*, *MGMT*, *MLH1*, *APC*, and *RUNX3* has been reported in GCs. Global DNA hypomethylation is frequently observed in GC cells [5]. Interestingly, the promoter CpG island hypermethylation has also been found both in the adjacent noncancerous tissues of patients with GC and in nonneoplastic gastric mucosae of subjects without GC. From the results of previous studies, the aberrant DNA methylation occurs in a gene-specific manner along the multistep gastric carcinogenesis [6].

MicroRNA (miRNA) is a small noncoding RNA molecule (containing approximately 21–23 nucleotides) that functions as RNA silencing and posttranscriptional regulation of gene expression. miRNAs can inhibit the translation of the target messenger RNAs (mRNAs) by binding to complementary sequences in the 30 untranslated regions (UTRs) of the mRNAs. Approximately more than one-third of human genes are known to be regulated by ~1000 miRNAs, and a single miRNA can regulate hundreds of unique mRNAs [7, 8]. miRNAs play important roles in the regulation of almost all biological processes, including proliferation, apoptosis, cell differentiation, metabolism, and epithelial-to-mesenchymal transition [9]. Therefore, miRNA dysregulation has been determined to correlate with cancer development and progression. The first report on miRNA dysregulation in cancer was in chronic lymphocytic leukemia [10]. To date, specific miRNAs have subsequently been found to have links with various malignancies including lung, colorectal, and breast cancers.

The role of miRNAs has also been addressed in gastric carcinogenesis. According to the results of miRNA chip studies, numerous miRNAs were demonstrated to be associated with GC. For example, miR-21, miR-17-92 cluster, miR-106b-25 cluster, and miR-150 were overexpressed in GC. However, miR-451, miR-141, miR-31, miR-218, and miR-9 were reported to be downregulated in GC tissue [11]. Alterations of miRNAs in gastric mucosa or blood or gastric juice may provide important data on early diagnosis and prognostication of GC [12]. More studies are warranted in the future.

24.2 *H. pylori*-Induced Gastric Carcinogenesis and Aberrant DNA Methylation

Helicobacter pylori (*H. pylori*) infection is an established risk factor for GC and is known to be associated with the accumulation of epigenetic alterations such as aberrant DNA methylation in gastric mucosa. A recent study on genome-wide methylation profiling shows that a number of genes were differentially methylated by *H. pylori* infection [13] (Fig. 24.1). Previous studies have shown that *H. pylori* infection can induce promoter hypermethylation of *CDKN2A* (*p16*), *CDH1*, and *MLH1* [4]. Since the aberrant DNA methylations of these genes are closely related to gastric carcinogenesis, *H. pylori* infection seems to be associated with promoter hypermethylation with gene type-specific methylation profiles in the multistep process of carcinogenesis [14, 15].

24.2.1 Underlying Mechanisms of Induction of Aberrant DNA Methylation by *H. pylori* Infection

Animal studies may address the underlying mechanisms regarding how *H. pylori*-induced chronic inflammation induces aberrant DNA methylation. If chronic active inflammation induced by *H. pylori* infection was suppressed by cyclosporin A in Mongolian gerbils, aberrant DNA methylation was significantly reduced, while the number of *H. pylori* was unaffected in gastric mucosae [16]. This study indicates that not *H. pylori* itself, but inflammation is important in the induction of aberrant DNA methylation [17]. However, the repeated induction of acute inflammation by ethanol or high salt diet could not induce aberrant DNA methylation [18]. *Il-1β*,

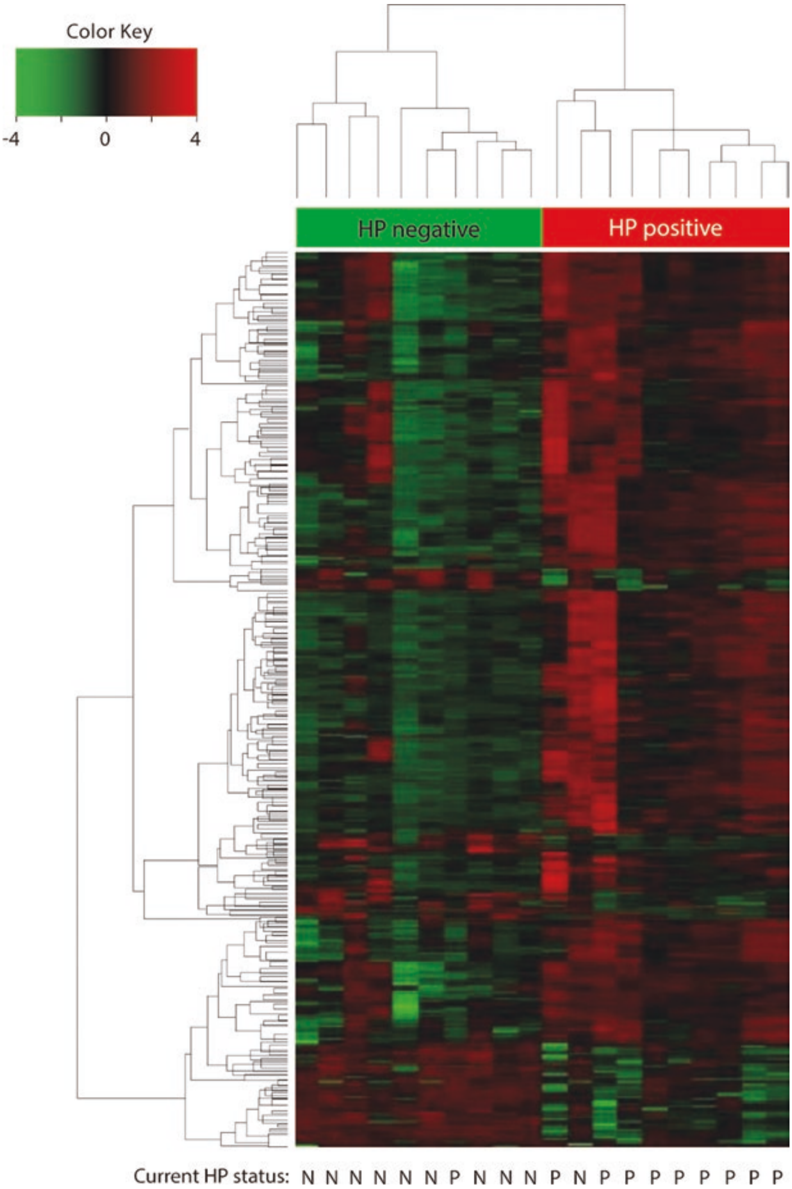


Fig. 24.1 Genome-wide DNA methylation profiles obtained from ten *H. pylori*-positive patients and ten *H. pylori*-positive controls. *HP Helicobacter pylori*, *N H. pylori*-negative, *P* current *H. pylori* infection-positive (adapted from Shin et al. [13])

Nos2, and *Tnf-α* were specifically upregulated by the *H. pylori*-induced inflammation. Probably, the chronic active *H. pylori* infection activates macrophage, and its secretion of interleukin (IL)-1β and tumor necrosis factor (TNF)-α, and the production of active oxygen species by nitric oxide synthase (NOS) may induce DMNT1 and in result aberrant DNA methylation in gastric mucosae [17].

24.2.2 Reversibility of Aberrant DNA Methylation Following *H. pylori* Eradication

To date, the changes in DNA methylation levels in gastric mucosae after anti-*H. pylori* treatment have not been clarified yet. Previous studies using nonquantitative methods have reported that hypermethylation in several tumor suppressor genes such as *CDH1* in gastric mucosae decreases following *H. pylori* eradication [19, 20]. However, more recent studies using quantitative methods have shown that aberrant DNA methylation induced by *H. pylori* seems to be partially reversible by *H. pylori* eradication [21, 22].

24.2.3 Epigenetic Fingerprint of *H. pylori* Infection and Epigenetic Field for Cancerization

In the viewpoint of the prediction for GC using methylation biomarkers, DNA methylation profiles obtained from noncancerous gastric mucosae may be useful in identifying a high-risk group for GC. As mentioned above, *H. pylori* infection is recognized as one of the most important risk factors for GC. As mucosal atrophy and intestinal metaplasia progress, however, the bacteria are slowly removed from the gastric mucosa and active inflammation gradually decreases. Thus, it is difficult to demonstrate a causal relationship between *H. pylori* infection and GC. From the epigenetic point of view, *H. pylori*-associated chronic inflammation is responsible for the promoter CpG island hypermethylation and global DNA hypomethylation. DNA hypermethylation caused by *H. pylori*-associated gastritis persists even after the disappearance of *H. pylori* (epigenetic fingerprint of *H. pylori* infection). The duration of *H. pylori* exposure and the epigenetic alterations induced by *H. pylori*, not *H. pylori* infection per se, are known to be correlated with the future risk for GC (epigenetic field for cancerization) [23] (Fig. 24.2). From this background, it has been suggested that the DNA methylation levels in the specific CpG loci obtained from blood or gastric mucosae or gastric

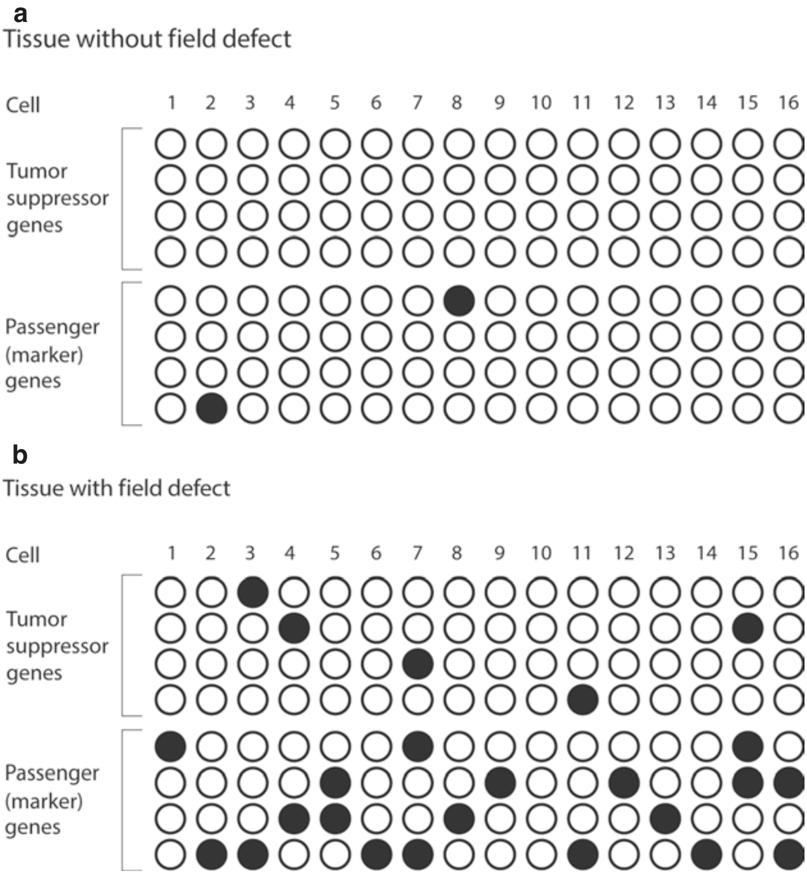


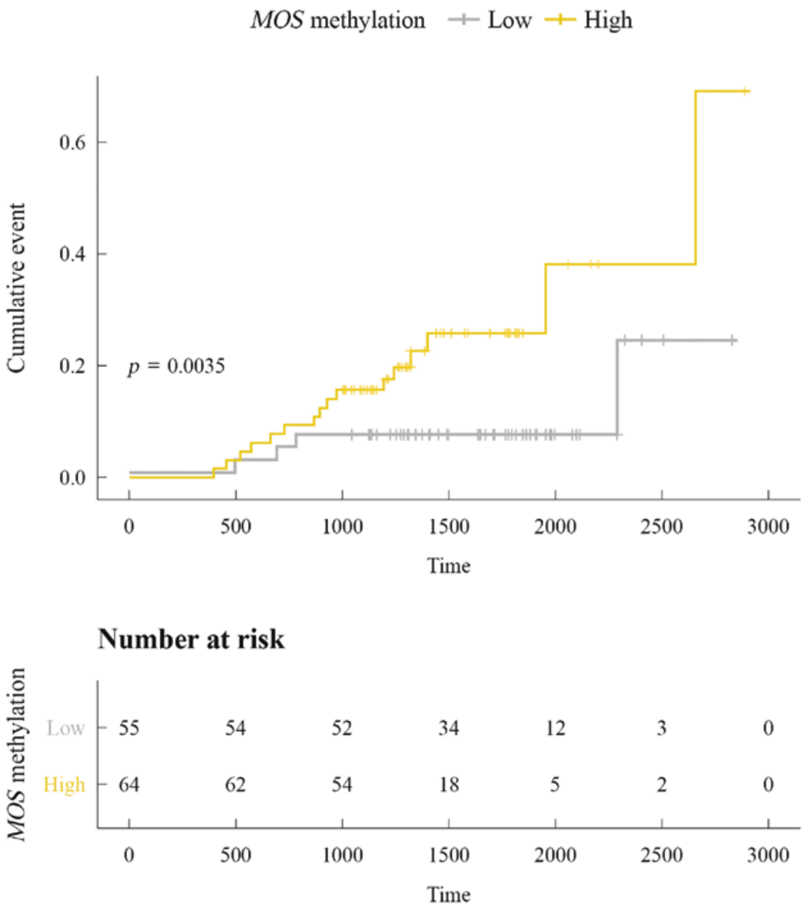
Fig. 24.2 Methylation of tumor suppressor and passenger (marker) genes. (**a**) Gastric mucosae without epigenetic field defect. (**b**) Gastric mucosae with field defect. In gastric mucosa with field defect, tumor suppressor genes may have low methylation levels, but passenger genes showed high levels of DNA methylation. Although methylation of passenger genes is not directly involved in carcinogenesis, their methylation levels correlate with those of tumor suppressor genes and reflect the future risk for gastric cancer. *Open circles* unmethylated genes, *closed circles* methylated (modified from Ushijima [4])

fluid might be used as a marker for GC. It has been suggested that the aberrant DNA methylation could be a surrogate marker for the risk of metachronous GC after endoscopic resection of early gastric cancer (EGC) [24, 25]. A recent study have reported that *MOS* methylation induced by *H. pylori* can be a promising marker for predicting metachronous recurrence after endoscopic resection of gastric neoplasms [26] (Fig. 24.3).

24.3 *H. pylori*-Induced Gastric Carcinogenesis and miRNA

The role of miRNAs in *H. pylori*-induced chronic inflammation has been evaluated in recent studies. According to a miRNA profiling study of 470 human miRNAs in noncancerous gastric mucosae of *H. pylori*-positive and *H. pylori*-negative subjects, a total of 30 miRNAs were significantly downregulated with *H. pylori* infection, while only one miRNA, miR-223, was upregulated with *H. pylori* infection [27]. Interestingly, the eradication of *H. pylori* normalized 14 of 30 miRNAs of which the expressions were downregulated [27]. One recent study has reported that 219 of 3523 miRNAs showed at least two-fold increased or decreased expressions in *H. pylori*-positive GC tissues compared with *H. pylori*-negative GC tissues [28]. According to a review article, miRNAs such as miR-17, miR-18a-8p, miR-20a, miR-21, miR-25, miR-146a, miR-155, miR-196, and miR-223 were upregulated both with *H. pylori* infection and GC, while miRNAs such as let-7a, miR-22, miR-31, miR-34b, miR-34c, miR-101, miR-140, miR-141, miR-203, miR-204, miR-210, miR-218, miR-375, miR-

Fig. 24.3 Kaplan-Meier curves for cumulative incidences of metachronous recurrence according to *MOS* methylation status. A higher level of *MOS* methylation was associated with an increased risk for metachronous recurrence after endoscopic resection of gastric neoplasms. The cutoff value (35.82%) of high level or low level of *MOS* methylation was determined by receiver operating characteristic curve analysis (modified from Shin et al. [26])



449, and miR-3178 were downregulated both with *H. pylori*-infected gastric mucosae and GC tissues [16, 29] (Tables 24.1 and 24.2). Similar to aberrant DNA methylation, miRNA alternations induced by *H. pylori* infection also can be a surrogate marker for predicting GC risk [24].

However, miRNAs that are dysregulated in response to *H. pylori* infection may not be the same miRNAs that are dysregulated in later stages of gastric carcinogenesis. For example, miR-106b is known as an oncogenic miRNA and is upregulated in GC, but its expression was reported to be suppressed in *H. pylori*-infected gastric mucosa [77, 78]. Likewise, miRNAs such as miR-34b, miR-34c, miR-103, miR-200a, miR-214, and miR-372 have been reported to be overexpressed in GC, but these are downregulated in *H. pylori*-infected gastric mucosa [16]. MiR-146a was reported to be upregulated in *H. pylori*-infected gastric mucosae [18, 67, 79]. However, its expression has been reported to be upregulated with GC in one study but downregulated in the other studies [80–83].

24.3.1 *H. pylori* and miRNA: Underlying Mechanisms

Underlying mechanisms are still unclear regarding how miRNAs dysregulated by *H. pylori* infection can be involved in the gastric carcinogenesis. Nevertheless, it appears to be related to (1) modulating host inflammatory immune response, (2) promoting cell-cycle progression, (3) inhibiting apoptosis and promoting proliferation, and (4) promoting invasion and metastasis of GC [16].

24.3.1.1 Modulation of Host Inflammatory Immune Response

H. pylori can dysregulate miRNA expression to evade host defenses and successfully persist in the gastric niche. For example, *H. pylori* upregulates the expressions of miR-146a and miR-155, both of which modulate the innate and adaptive immune responses in a nuclear factor (NF)-κB-dependent manner [18, 66, 70, 71]. MiR-146a targets the Toll-like receptor (TLR) signaling adaptor molecules, interleukin-1 receptor-associated kinase (IRAK1), and TNF receptor-associated factor (TRAF6) [18, 66]. MiR-155 targets the myeloid differentiation primary response gene (MyD88), the universal adaptor protein used by TLRs to activate NF-κB [70, 71]. As a result, both miR-146a overexpression and miR-155 overexpression negatively regulate *H. pylori*-induced IL-8, TNF-α, IL-1β, growth-related oncogene (GRO)-α, and macrophage inflammatory protein (MIP)-3α expression, all key components to the pro-inflammatory innate and adaptive immune responses [16].

24.3.1.2 Promotion of Cell-Cycle Progression

Several miRNAs dysregulated by *H. pylori* infection promote cell-cycle progression by upregulating cyclin expression and/or downregulating expression of cyclin-dependent kinase (CDK) inhibitors (p15, p16, p18, p19, p21, p27, p28, p57) in GC [16]. The cell cycle consists of four distinct phases: G1, S, G2, and M. Two key classes of regulatory molecules, cyclins and CDKs, determine a cell's progress through the cell cycle. CDK inhibitors prevent the progression of the cell cycle and function as tumor suppressors. miRNAs such as miR-106b and miR-449 target cyclins and CDKs as well as CDK inhibitors to disrupt normal cell-cycle progression [41, 58, 59]. *H. pylori* is believed to modulate the expressions of cyclins, CDKs, and CDK inhibitors through the dysregulation of host miRNAs. Thus, it may induce gastric carcinogenesis [16].

24.3.1.3 Inhibition of Apoptosis and Promotion of Proliferation

miRNA dysregulation induced by *H. pylori* infection inhibits apoptosis and promotes cell survival. For example, miR-21 is overexpressed in GC tissues and cell lines, as well as in *H. pylori*-infected noncancerous gastric mucosae. It was also upregulated in cultured gastric epithelial cell lines cocultured with *H. pylori* [63]. Overexpression of miR-21 promoted cell

Table 24.1 MicroRNAs downregulated in response to *Helicobacter pylori*

MicroRNAs	Downregulated in gastric cancer	Target mRNAs	Biological process targeted	Reference
let-7a	Yes	RAB40C	Cell-cycle progression, proliferation	[27, 30, 31]
		HMGA2	Invasion	
let-7b		HMGA2	Invasion	[27, 30, 32, 33]
		TLR4, NF-κB, COX-2, cyclin D1, IL1B	Immune response	
let-7d		HMGA2	Invasion	[27, 30]
let-7e		HMGA2	Invasion	[27, 30]
let-7f		HMGA2	Invasion	[27, 30]
miR-1		ND	Proliferation	[34]
miR-22	Yes	NLRP3	Proliferation	[35]
miR-31	Yes	ND	ND	[27]
miR-32		ND	ND	[27]
miR-34b	Yes	ND	ND	[36, 37]
miR-34c	Yes	ND	ND	[36, 38]
miR-101	Yes	COX-2, FOS	Proliferation	[27, 39, 40]
		MCL1	Apoptosis	
		EZH2	Invasion, migration	
miR-103		ND	ND	[27]
miR-106b		p21	Cell-cycle progression	[27, 41, 42]
			Proliferation	
		BIM	Apoptosis	
miR-125a		ERBB2	Proliferation	[27, 43]
miR-130a		ND	ND	[27]
miR-133		ND	Proliferation	[34]
miR-140	Yes	PD-L1	Suppress GC by targeting the immune checkpoint Molecule PD-L1	[44]
miR-141	Yes	FGFR2	Proliferation	[27, 45]
miR-200a		ZEB1, ZEB2	EMT	[27, 46, 47]
miR-200b		BCL2, XIAP	Apoptosis	[27, 46–48]
		ZEB1, ZEB2	EMT	

(continued)

Table 24.1 (continued)

MicroRNAs	Downregulated in gastric cancer	Target mRNAs	Biological process targeted	Reference
miR-200c		BCL2, XIAP	Apoptosis	[27, 47, 48]
			EMT	
miR-203	Yes	ABL1	Proliferation	[27, 49]
			Invasion	
miR-204	Yes	EZR, BIRC2	Proliferation	[27, 50, 51]
miR-210	Yes	ND	ND	[27]
miR-214		ND	ND	[27]
miR-218	Yes	ECOP	Proliferation	[52, 53]
			Apoptosis	
		ROBO1	Invasion, metastasis	
miR-320		ND	ND	[27]
miR-370		FOXM1	Proliferation	[54]
miR-371-5p		LATS2	Cell-cycle progression	[55]
miR-372		LATS2	Cell-cycle progression	[55]
miR-373		LATS2	Cell-cycle progression	[55]
miR-375	Yes	PDK1, 14-3-3	Apoptosis	[27, 29, 56, 57]
		JAK2	Proliferation	
miR-377		ND	ND	[27]
miR-379		ND	ND	[27]
miR-429		BCL2, XIAP	Apoptosis	[27, 48, 50]
		MYC	Proliferation	
miR-449	Yes	GMNN, CCNE2, MET, SIRT1	Cell-cycle progression	[58, 59]
miR-455		ND	ND	[27]
miR-491-5p		ND	ND	[27]
miR-500		ND	ND	[27]
miR-532		ND	ND	[27]
miR-652		ND	ND	[27]
miR-3178	Yes	ECOP	Proliferation	[29, 60]
			Apoptosis	

Modified from Noto et al. [16]

EMT Epithelial-to-mesenchymal transition, *ND* Target mRNA or biological process not determined

Table 24.2 MicroRNAs upregulated in response to *Helicobacter pylori*

MicroRNAs	Upregulated in gastric cancer	Target mRNAs	Biological process targeted	Reference
miR-17	Yes	p21	Cell-cycle progression	[61]
miR-18a-3p	Yes	BZRAP1	Proliferation	[62]
miR-20a	Yes	p21	Cell-cycle progression	[61]
miR-21	Yes	PTEN, PDCD4	Apoptosis	[63, 64]
		RECK	Metastasis, angiogenesis	
miR-25	Yes	ND	ND	[64, 65]
miR-93		ND	ND	[64]
miR-146a	Yes	IRAK1,TRAF6	Immune response	[18, 66, 67]
			Proliferation	
		SMAD4	Apoptosis	
miR-155	Yes	IKK-ε, SMAD2	Immune response	[29, 68–71]
		FADD, PKIα	Apoptosis	
miR-194		ND	ND	[64]
miR-196	Yes	ND	ND	[64, 72, 73]
miR-200b		ZEB1	EMT	[74, 75]
miR-200c		ZEB1	EMT	[32, 74]
		ND	ND	
miR-222		RECK	Metastasis, angiogenesis	[76]
miR-223	Yes	EPB41L3	Invasion, metastasis	[27, 29, 69]
miR-584		PPP2a, FOXA1	EMT	[48]
miR-1290		NKRF, FOXA1	EMT	[48]

Modified from Noto et al. [16]
EMT Epithelial-to-mesenchymal transition, *ND* Target mRNA or biological process not determined

proliferation and migration and inhibited apoptosis in this cell line. Activator protein (AP)-1 and the signal transducer and activator of transcription 3 (STAT3) can induce the expression of miR-21. *H. pylori* infection induces NF-κB and IL-6 secretion in gastric mucosae, which activate AP-1 and STAT3, respectively, which explains the upregulation of miR-21 during *H. pylori* infection. In addition, miR-21 targets phosphatase and tensin homolog (PTEN) and programmed cell death protein 4 (PDCD4) [30, 84–86].

24.3.1.4 Promotion of Tumor Invasion and Metastasis

H. pylori-induced dysregulation of specific miRNA may play a role in angiogenesis, invasion, and metastasis of GC. For example, *H. pylori* infection negatively regulates miR-449 during gastric carcinogenesis, which leads to the upregulation of Met, a known proto-oncogene. The upregulation of Met is known to promote not only proliferation but also angiogenesis, invasion, and metastasis of cancer [58, 59]. MiR-218 is known to be inhibited in GC, in relation to invasion and metastasis [87]. It might be attributed to the roundabout homolog 1 (ROBO1) signaling pathway that has been implicated in the regulation of cell migration [53]. As mentioned above, *H. pylori* infection induces overexpression of miR-21. miR-21 has been reported to enhance the invasiveness of GC cells, which is attributed to inhibit reversion-inducing cysteine-rich protein with Kazal motifs (RECK), a tumor and metastasis suppressor that inhibits tumor metastasis and angiogenesis through modulation of matrix metalloproteinases (MMPs) [63]. *H. pylori* infection is known to induce the expression of MMPs, including MMP-1, MMP-2, MMP-3, MMP-7, and MMP-9 [88]. So, *H. pylori* has the potential to modulate the expression of MMPs through the dysregulation of host miRNAs [16].

24.4 Conclusions

The aberrant DNA methylation and the dysregulation of numerous miRNAs by chronic active *H. pylori* infection play an important role in gastric carcinogenesis. Alterations of these epigenetic changes in gastric mucosae or biological fluids have diagnostic and therapeutic potentials in GC, but further studies are necessary to validate these potentials in large clinical trials.

References

1. Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007;128:683–92.
2. McCabe MT, Brandes JC, Vertino PM. Cancer DNA methylation: molecular mechanisms and clinical implications. Clin Cancer Res. 2009;15:3927–37.
3. Stricker T, Catenacci DV, Seiwert TY. Molecular profiling of cancer—the future of personalized cancer medicine: a primer on cancer biology and the tools necessary to bring molecular testing to the clinic. Semin Oncol. 2011;38:173–85.
4. Ushijima T. Epigenetic field for cancerization. J Biochem Mol Biol. 2007;40:142–50.
5. Park SY, Yoo EJ, Cho NY, Kim N, Kang GH. Comparison of CpG Island hypermethylation and repetitive DNA hypomethylation in premalignant stages of gastric cancer, stratified for *Helicobacter pylori* infection. J Pathol. 2009;219:410–6.
6. Kang GH, Shim YH, Jung HY, Kim WH, Ro JY, Rhyu MG. CpG Island methylation in premalignant stages of gastric carcinoma. Cancer Res. 2001;61:2847–51.
7. Nelson KM, Weiss GJ. MicroRNAs and cancer: past, present, and potential future. Mol Cancer Ther. 2008;7:3655–60.
8. Sontheimer EJ, Carthew RW. Silence from within: endogenous siRNAs and miRNAs. Cell. 2005;122:9–12.
9. Ambros V. The functions of animal microRNAs. Nature. 2004;431:350–5.
10. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A. 2002;99:15,524–9.
11. Song B, Ju J. Impact of miRNAs in gastrointestinal cancer diagnosis and prognosis. Expert Rev Mol Med. 2010;12:e33.
12. Toiyama Y, Okugawa Y, Goel A. DNA methylation and microRNA biomarkers for noninvasive detection of gastric and colorectal cancer. Biochem Biophys Res Commun. 2014;455:43–57.
13. Shin CM, Kim N, Jung Y, Park JH, Kang GH, Park WY, et al. Genome-wide DNA methylation profiles in noncancerous gastric mucosae with regard to *Helicobacter pylori* infection and the presence of gastric cancer. Helicobacter. 2011;16:179–88.
14. Nakajima T, Yamashita S, Maekita T, Niwa T, Nakazawa K, Ushijima T. The presence of a methylation fingerprint of *Helicobacter pylori* infection in human gastric mucosae. Int J Cancer. 2009;124:905–10.
15. Shin CM, Kim N, Jung Y, Park JH, Kang GH, Kim JS, et al. Role of *Helicobacter pylori* infection in aberrant DNA methylation along multistep gastric carcinogenesis. Cancer Sci. 2010;101:1337–46.
16. Noto JM, Peek RM. The role of microRNAs in *Helicobacter pylori* pathogenesis and gastric carcinogenesis. Front Cell Infect Microbiol. 2011;1:21.
17. Chiba T, Marusawa H, Ushijima T. Inflammation-associated cancer development in digestive organs: mechanisms and roles for genetic and epigenetic modulation. Gastroenterology. 2012;143:550–63.
18. Liu Z, Xiao B, Tang B, Li B, Li N, Zhu E, et al. Up-regulated microRNA-146a negatively modulate *Helicobacter pylori*-induced inflammatory response in human gastric epithelial cells. Microbes Infect. 2010;12:854–63.
19. Chan AO, Peng JZ, Lam SK, Lai KC, Yuen MF, Cheung HK, et al. Eradication of *Helicobacter pylori* infection reverses E-cadherin promoter hypermethylation. Gut. 2006;55:463–8.
20. Perri F, Cotugno R, Piepoli A, Merla A, Quitadamo M, Gentile A, et al. Aberrant DNA methylation in non-neoplastic gastric mucosa of *H. pylori* infected patients and effect of eradication. Am J Gastroenterol. 2007;102:1361–71.
21. Nakajima T, Enomoto S, Yamashita S, Ando T, Nakanishi Y, Nakazawa K, et al. Persistence of a component of DNA methylation in gastric mucosae after *Helicobacter pylori* eradication. J Gastroenterol. 2010;45:37–44.
22. Shin CM, Kim N, Lee HS, Park JH, Ahn S, Kang GH, et al. Changes in aberrant DNA methylation after *Helicobacter pylori* eradication: a long-term follow-up study. Int J Cancer. 2013;133:2034–42.
23. Ushijima T, Hattori N. Molecular pathways: involvement of *Helicobacter pylori*-triggered inflammation in the formation of an epigenetic field defect, and its usefulness as cancer risk and exposure markers. Clin Cancer Res. 2012;18:923–9.
24. Grady WM, Yu M, Markowitz SD. Epigenetic alterations in the gastrointestinal tract: current and emerging use for biomarkers of cancer. Gastroenterology. 2021;160:690–709.
25. Maeda M, Nakajima T, Oda I, Shimazu T, Yamamichi N, Maekita T, et al. High impact of methylation accumulation on metachronous gastric cancer: 5-year follow-up of a multicentre prospective cohort study. Gut. 2017;66:1721–3.
26. Shin CM, Kim N, Yoon H, Choi YJ, Park JH, Park YS, et al. Aberrant DNA methylation maker for predicting metachronous recurrence after endoscopic

resection of gastric neoplasms. Cancer Res Treat. 2022;54:1157–66.

27. Matsushima K, Isomoto H, Inoue N, Nakayama T, Hayashi T, Nakayama M, et al. MicroRNA signatures in *Helicobacter pylori*-infected gastric mucosa. Int J Cancer. 2011;128:361–70.
28. Chang H, Kim N, Park JH, Nam RH, Choi YJ, Lee HS, et al. Different microRNA expression levels in gastric cancer depending on *Helicobacter pylori* infection. Gut Liver. 2015;9:188–96.
29. Liu AR, Yan ZW, Jiang LY, Lv Z, Li YK, Wang BG. The role of non-coding RNA in the diagnosis and treatment of *Helicobacter pylori*-related gastric cancer, with a focus on inflammation and immune response. Front Med (Lausanne). 2022;9:1009021.
30. Motoyama K, Inoue H, Nakamura Y, Uetake H, Sugihara K, Mori M. Clinical significance of high mobility group A2 in human gastric cancer and its relationship to let-7 microRNA family. Cancer Res. 2008;14:2334–40.
31. Yang Q, Jie Z, Cao H, Greenlee AR, Yang C, Zou F, et al. Low-level expression of let-7a in gastric cancer and its involvement in tumorigenesis by targeting RAB40C. Carcinogenesis. 2011;32:713–22.
32. Isomoto H, Matsushima K, Inoue N, Hayashi T, Nakayama T, Kunizaki M, et al. Interweaving microRNAs and proinflammatory cytokines in gastric mucosa with reference to *H. pylori* infection. J Clin Immunol. 2012;32:290–9.
33. Teng GG, Wang WH, Dai Y, Wang SJ, Chu YX, Li J. Let-7b is involved in the inflammation and immune responses associated with *Helicobacter pylori* infection by targeting toll-like receptor 4. PLoS One. 2013;8:e56709.
34. Saito Y, Suzuki H, Tsugawa H, Suzuki S, Matsuzaki J, Hirata K, et al. Dysfunctional gastric emptying with down-regulation of muscle-specific microRNAs in *Helicobacter pylori*-infected mice. Gastroenterology. 2011;140:189–98.
35. Li S, Liang X, Ma L, Shen L, Li T, Zheng L, et al. MiR-22 sustains NLRP3 expression and attenuates *H. pylori*-induced gastric carcinogenesis. Oncogene. 2018;37:884–96.
36. Suzuki H, Yamamoto E, Nojima M, Kai M, Yamano HO, Yoshikawa K, et al. Methylation-associated silencing of microRNA-34b/c in gastric cancer and its involvement in an epigenetic field defect. Carcinogenesis. 2010;31:2066–73.
37. Tsai KW, Wu CW, Hu LY, Li SC, Liao YL, Lai CH, et al. Epigenetic regulation of miR-34b and miR-129 expression in gastric cancer. Int J Cancer. 2011;129:2600–10.
38. Wang AM, Huang TT, Hsu KW, Huang KH, Fang WL, Yang MH, et al. Yin Yang 1 is a target of microRNA-34 family and contributes to gastric carcinogenesis. Oncotarget. 2014;5:5002–16.
39. Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, et al. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science. 2008;322:1695–9.
40. Wang HJ, Ruan HJ, He XJ, Ma YY, Jiang XT, Xia YJ, et al. MicroRNA-101 is down-regulated in gastric cancer and involved in cell migration and invasion. Eur J Cancer. 2010;46:2295–303.
41. Kan T, Sato F, Ito T, Matsumura N, David S, Cheng Y, et al. The miR-106b-25 polycistron, activated by genomic amplification, functions as an oncogene by suppressing p21 and Bim. Gastroenterology. 2009;136:1689–700.
42. Petrocca F, Visone R, Onelli MR, Shah MH, Nicoloso MS, de Martino I, et al. E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer. Cancer Cell. 2008;13: 272–86.
43. Nishida N, Mimori K, Fabbri M, Yokobori T, Sudo T, Tanaka F, et al. MicroRNA-125a-5p is an independent prognostic factor in gastric cancer and inhibits the proliferation of human gastric cancer cells in combination with trastuzumab. Clin Cancer Res. 2011;17:2725–33.
44. Zhao M, Liu Q, Liu W, Zhou H, Zang X, Lu J. MicroRNA140 suppresses *Helicobacter pylori* positive gastric cancer growth by enhancing the antitumor immune response. Mol Med Rep. 2019;20:2484–92.
45. Du Y, Xu Y, Ding L, Yao H, Yu H, Zhou T, et al. Down-regulation of miR-141 in gastric cancer and its involvement in cell growth. J Gastroenterol. 2009;44:556–61.
46. Ahn SM, Cha JY, Kim J, Kim D, Trang HT, Kim YM, et al. Smad3 regulates E-cadherin via miRNA-200 pathway. Oncogene. 2012;31:3051–9.
47. Shinozaki A, Sakatani T, Ushiku T, Hino R, Isogai M, Ishikawa S, et al. Downregulation of microRNA-200 in EBV-associated gastric carcinoma. Cancer Res. 2010;70:4719–27.
48. Zhu W, Xu H, Zhu D, Zhi H, Wang T, Wang J, et al. miR-200bc/429 cluster modulates multidrug resistance of human cancer cell lines by targeting BCL2 and XIAP. Cancer Chemother Pharmacol. 2012;69:723–31.
49. Craig VJ, Cogliatti SB, Rehrauer H, Wündisch T, Müller A. Epigenetic silencing of microRNA-203 dysregulates ABL1 expression and drives *Helicobacter*-associated gastric lymphomagenesis. Cancer Res. 2011;71:3616–24.
50. Lam EK, Wang X, Shin VY, Zhang S, Morrison H, Sun J, et al. A microRNA contribution to aberrant Ras activation in gastric cancer. Am J Transl Res. 2011;3:209–18.
51. Chen P, Guo H, Wu X, Li J, Duan X, Ba Q, et al. Epigenetic silencing of microRNA-204 by *Helicobacter pylori* augments the NF-kappaB signaling pathway in gastric cancer development and progression. Carcinogenesis. 2020;41:430–41.
52. Gao C, Zhang Z, Liu W, Xiao S, Gu W, Lu H. Reduced microRNA-218 expression is associated with high nuclear factor kappa B activation in gastric cancer. Cancer. 2010;116:41–9.
53. Tie J, Pan Y, Zhao L, Wu K, Liu J, Sun S, et al. MiR-218 inhibits invasion and metastasis of gastric

cancer by targeting the Robo1 receptor. PLoS Genet. 2010;6:e1000879.

54. Feng Y, Wang L, Zeng J, Shen L, Liang X, Yu H, et al. FoxM1 is overexpressed in *Helicobacter pylori*-induced gastric carcinogenesis and is negatively regulated by miR-370. Mol Cancer Res. 2013;11: 834–44.
55. Belair C, Baud J, Chabas S, Sharma CM, Vogel J, Staedel C, et al. *Helicobacter pylori* interferes with an embryonic stem cell micro RNA cluster to block cell cycle progression. Silence. 2011;2:7.
56. Ding L, Xu Y, Zhang W, Deng Y, Si M, Du Y, et al. MiR-375 frequently downregulated in gastric cancer inhibits cell proliferation by targeting JAK2. Cell Res. 2010;20:784–93.
57. Tsukamoto Y, Nakada C, Noguchi T, Tanigawa M, Nguyen LT, Uchida T, et al. MicroRNA-375 is downregulated in gastric carcinomas and regulates cell survival by targeting PDK1 and 14-3-3zeta. Cancer Res. 2010;70:2339–49.
58. Bou Kheir T, Futoma-Kazmierczak E, Jacobsen A, Krogh A, Bardram L, Hother C, et al. miR-449 inhibits cell proliferation and is down-regulated in gastric cancer. Mol Cancer. 2011;10:29.
59. Lize M, Klimke A, Dobbelstein M. MicroRNA-449 in cell fate determination. Cell Cycle. 2011;10:2874–82.
60. Zou M, Wang F, Jiang A, Xia A, Kong S, Gong C, et al. MicroRNA-3178 ameliorates inflammation and gastric carcinogenesis promoted by *Helicobacter pylori* new toxin, Tip-alpha, by targeting TRAF3. Helicobacter. 2017;22:e12348.
61. Saito Y, Murata-Kamiya N, Hirayama T, Ohba Y, Hatakeyama M. Conversion of *Helicobacter pylori* CagA from senescence inducer to oncogenic driver through polarity-dependent regulation of p21. J Exp Med. 2010;207:2157–74.
62. Tsai CC, Chen TY, Tsai KJ, Lin MW, Hsu CY, Wu DC, et al. NF-kappaB/miR18a-3p and miR-4286/BZRAP1 axis may mediate carcinogenesis in *Helicobacter pylori*-associated gastric cancer. Biomed Pharmacother. 2020;132:110869.
63. Zhang Z, Li Z, Gao C, Chen P, Chen J, Liu W, et al. miR-21 plays a pivotal role in gastric cancer pathogenesis and progression. Lab Investig. 2008;88:1358–66.
64. Shiotani A, Uedo N, Iishi H, Murao T, Kanzaki T, Kimura Y, et al. *H. pylori* eradication did not improve dysregulation of specific oncogenic miRNAs in intestinal metaplastic glands. J Gastroenterol. 2012;47:988–98.
65. Li BS, Zuo QF, Zhao YL, Xiao B, Zhuang Y, Mao XH, et al. MicroRNA-25 promotes gastric cancer migration, invasion and proliferation by directly targeting transducer of ERBB2, 1 and correlates with poor survival. Oncogene. 2015;34:2556–65.
66. Li N, Xu X, Xiao B, Zhu ED, Li BS, Liu Z, et al. *H. pylori* related proinflammatory cytokines contribute to the induction of miR-146a in human gastric epithelial cells. Mol Biol Rep. 2012;39:4655–61.
67. Xiao B, Zhu ED, Li N, Lu DS, Li W, Li BS, et al. Increased miR-146a in gastric cancer directly targets SMAD4 and is involved in modulating cell proliferation and apoptosis. Oncol Rep. 2012;27:559–66.
68. Fassi Fehri L, Koch M, Belogolova E, Khalil H, Bolz C, Kalali B, et al. *Helicobacter pylori* induces miR-155 in T cells in a cAMP-Foxp3-dependent manner. PLoS One. 2010;5:e9500.
69. Oertli M, Engler DB, Kohler E, Koch M, Meyer TF, Müller A. MicroRNA-155 is essential for the T cell-mediated control of *Helicobacter pylori* infection and for the induction of chronic Gastritis and Colitis. J Immunol. 2011;187:3578–86.
70. Tang B, Xiao B, Liu Z, Li N, Zhu ED, Li BS, et al. Identification of MyD88 as a novel target of miR-155, involved in negative regulation of *Helicobacter pylori*-induced inflammation. FEBS Lett. 2010;584:1481–6.
71. Xiao B, Liu Z, Li BS, Tang B, Li W, Guo G, et al. Induction of microRNA-155 during *Helicobacter pylori* infection and its negative regulatory role in the inflammatory response. J Infect Dis. 2009;200:916–25.
72. Sun M, Liu XH, Li JH, Yang JS, Zhang EB, Yin DD, et al. MiR-196a is upregulated in gastric cancer and promotes cell proliferation by downregulating p27(kip1). Mol Cancer Ther. 2012;11:842–52.
73. Liao YL, Hu LY, Tsai KW, Wu CW, Chan WC, Li SC, et al. Transcriptional regulation of miR-196b by ETS2 in gastric cancer cells. Carcinogenesis. 2012;33:760–9.
74. Baud J, Varon C, Chabas S, Chambonnier L, Darfeuille F, Staedel C. *Helicobacter pylori* initiates a mesenchymal transition through ZEB1 in gastric epithelial cells. PLoS One. 2013;8:e60315.
75. Kurashige J, Mima K, Sawada G, Takahashi Y, Eguchi H, Sugimachi K, et al. Epigenetic modulation and repression of miR-200b by cancer-associated fibroblasts contribute to cancer invasion and peritoneal dissemination in gastric cancer. Carcinogenesis. 2015;36:133–41.
76. Li N, Tang B, Zhu ED, Li BS, Zhuang Y, Yu S, et al. Increased miR-222 in *H. pylori*-associated gastric cancer correlated with tumor progression by promoting cancer cell proliferation and targeting RECK. FEBS Lett. 2012;586:722–8.
77. Yu D, Shin HS, Lee YS, Lee YC. miR-106b modulates cancer stem cell characteristics through TGF-beta/Smad signaling in CD44-positive gastric cancer cells. Lab Investig. 2014;94:1370–81.
78. Ye F, Tang C, Shi W, Qian J, Xiao S, Gu M, et al. A MDM2-dependent positive-feedback loop is involved in inhibition of miR-375 and miR-106b induced by *Helicobacter pylori* lipopolysaccharide. Int J Cancer. 2015;136:2120–31.
79. Li X, Zhang Y, Shi Y, Dong G, Liang J, Han Y, et al. MicroRNA-107, an oncogene microRNA that regulates tumour invasion and metastasis by targeting DICER1 in gastric cancer. J Cell Mol Med. 2011;15:1887–95.
80. Crone SG, Jacobsen A, Federspiel B, Bardram L, Krogh A, Lund AH, et al. microRNA-146a inhibits G protein-coupled receptor-mediated activation of

NF-kappaB by targeting CARD10 and COPS8 in gastric cancer. Mol Cancer. 2012;11:71.
81. Zhou L, Zhao X, Han Y, Lu Y, Shang Y, Liu C, et al. Regulation of UHRF1 by miR-146a/b modulates gastric cancer invasion and metastasis. FASEB J. 2013;27:4929–39.
82. Kogo R, Mimori K, Tanaka F, Komune S, Mori M. Clinical significance of miR-146a in gastric cancer cases. Clin Cancer Res. 2011;17:4277–84.
83. Yao Q, Cao Z, Tu C, Zhao Y, Liu H, Zhang S. MicroRNA-146a acts as a metastasis suppressor in gastric cancer by targeting WASF2. Cancer Lett. 2013;335:219–24.
84. Kang YH, Lee HS, Kim WH. Promoter methylation and silencing of PTEN in gastric carcinoma. Lab Investig. 2002;82:285–91.
85. Lu Z, Liu M, Stribinskis V, Klinge CM, Ramos KS, Colburn NH, et al. MicroRNA-21 promotes cell transformation by targeting the programmed cell death 4 gene. Oncogene. 2008;27:4373–9.
86. Yamanaka Y, Tagawa H, Takahashi N, Watanabe A, Guo YM, Iwamoto K, et al. Aberrant overexpression of microRNAs activate AKT signaling via down-regulation of tumor suppressors in natural killer-cell lymphoma/leukemia. Blood. 2009;114:3265–75.
87. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A. 2006;103:2257–61.
88. Elkington PT, O’Kane CM, Friedland JS. The paradox of matrix metalloproteinases in infectious disease. Exp Immunol. 2005;142:12–20.

Gastric Cancer: *H. pylori* and Macrophage Migration Inhibitory Factor

25

Kichul Yoon

Abstract

Macrophage migration inhibitory factor (MIF) is known to have an important role in angiogenesis, lymph node metastasis, tumor invasion, and distant metastasis according to various studies. As MIF has been reported to be upregulated in gastric cancer (GC) epithelial cell, the measurement of the cytokine might be helpful for the diagnosis and the prediction of prognosis. *Helicobacter pylori* (*H. pylori*) is reported to promote the proliferation of GC cell line via the stimulation of MIF expression; however, the contradictory studies necessitate further investigation. The possible factors that could link *H. pylori* and MIF are cytokine receptor CD74, interleukin (IL)-6, cytokines for angiogenesis, reactive oxygen species, and autophagy. The role of MIF in carcinogenesis of GC in relation to *H. pylori* could be a potential research topic.

Keywords

Gastric cancer · *Helicobacter pylori* · Macrophage migration inhibitory factor

25.1 Introduction

The tumor microenvironment has become an important concept in explaining tumorigenesis. Among the factors known to be involved, macrophage migration inhibitory factor (MIF) has been shown to be related to various types of malignancy such as esophageal cancer, ovarian cancer, hepatocellular carcinoma, nonsmall-cell lung cancer, and prostate cancer [1–3]. MIF has been reported to have a role in angiogenesis, lymph node metastasis, and distant metastasis [2, 4, 5]. An increased epithelial and serum expression of MIF in gastric cancer (GC) suggested its role in the diagnosis and prediction of prognosis. MIF as a possible link between *H. pylori* and GC is described here.

25.2 Introduction of Macrophage Migration Inhibitory Factor

MIF is one of the first cytokines that have ever been discovered. It was described as a certain T-lymphocyte-derived factor that could inhibit the migration of monocyte and macrophage. Thereafter, MIF has been shown to be related to delayed-type hypersensitivity. As the three-dimensional structure of MIF was revealed, CD74—the receptor for MIF—was cloned, and the co-receptor CD44 was found [6–8]. MIF is

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involved in signal transduction, and it continuously stimulates ERK1 and ERK2 MAP kinase, which property was related to carcinogenesis [9]. In addition to the important role in innate immunity, MIF-related continuous and repetitive inflammation has been associated with tumorigenesis as well [5].

25.3 Role of Macrophage Migration Inhibitory Factor in Tumorigenesis and Tumor Progression

There have been various pieces of evidence supporting the role of MIF in tumorigenesis and tumor progression. As shown in Table 25.1, the relationship between MIF and cancer such as non-small-cell lung cancer, breast cancer, colorectal cancer, prostate cancer, esophageal cancer, hepatocellular carcinoma, and ovarian cancer has been suggested [10–16]. In GC, the activity of MIF increases during the progression from normal epithelium to carcinoma [17–19]. Figure 25.1 shows the possible molecular biological pathway of MIF involved in tumorigenesis. MIF has a role in cell proliferation via signaling cascade, especially through Ras-related pathway. MIF also negatively affects the well-known tumor suppressor p53 by inhibiting its antiproliferative property and its role in apoptosis. The high concentration of MIF expressed by dysplastic or inflammatory cells lets the microenvironment bypass the p53 pathway, cumulating mutations via cellular proliferation, elongation of cellular life span, and inhibition of cell death [2, 5, 19, 37–42]. MIF inhibits activity of p21, cyclin G1, and Mdm2 [9]. MIF-deficient cells did not proliferate following the exposure to tumorigenic stimuli; it could be related to E2F and p53 tumor suppressor RB [43]. In Eμ-Myc lymphoma model, MIF deficiency led to delayed lymphomagenesis [44]. The angiogenetic activity of MIF is well known. In B cell lymphoma animal model,

Table 25.1 MIF and cancer

Cancer	MIF	References
Nonsmall-cell lung cancer	Angiogenesis	[12, 20]
Breast cancer	Angiogenesis, interaction between breast cancer cells and stroma, autophagy regulation	[4, 11, 21]
Colon cancer	Promotor of the cancer; determinant of hypoxia-induced apoptosis	[22, 23]
Prostate cancer	Upregulated in prostate cancer	[24–28]
Esophageal cancer	Differentiation and lymph node status	[15]
Ovarian cancer	Prognostic factor; secreted from cancer cells	[13, 29, 30]
Hepatocellular carcinoma	Metastasis, angiogenesis	[2, 14]
Stomach cancer	Progressive increase from normal cells to cancer	[17, 18, 31–36]

MIF Macrophage migration inhibitory factor

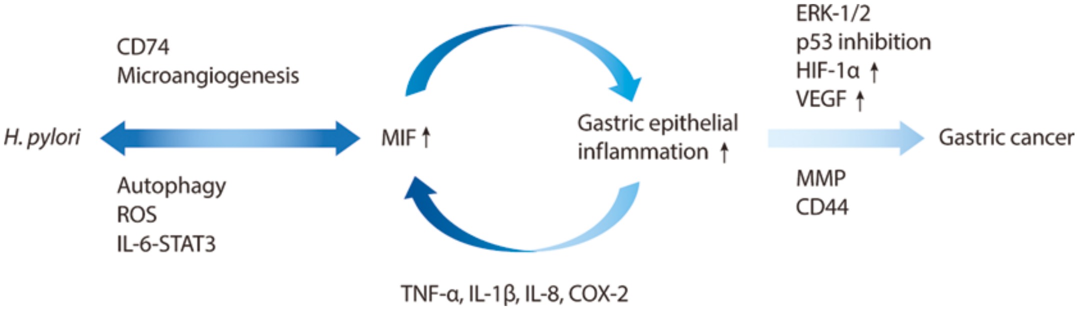

Fig. 25.1 Possible connection between *H. pylori*, macrophage migration inhibitory factor (*MIF*), and gastric cancer

the administration of anti-MIF antibody inhibited tumor growth; especially, the angiogenesis was affected [45]. Similar results were shown in nonsmall-cell lung cancer and colorectal cancer models due to the interaction between MIF and CXC chemokine, interleukin (IL)-8, or vascular endothelial growth factor (VEGF) [2, 20].

MIF also enhances cell proliferation by upregulating HIF-1 and tumor invasion through matrix metalloproteinase. Its role in chronic inflammation has been associated with the receptor CD74 and co-receptor CD44 [3, 5, 18, 19, 37–39, 41].

25.4 *H. pylori* and Macrophage Migration Inhibitory Factor

The relationship between *H. pylori* and MIF has been sought in various aspects including cell line research and clinical study with human tissue. An increased expression of MIF by epithelial cells, T cells, and macrophages in stomach mucosa was reported to be associated with *H. pylori* infection. The difference in the distribution of MIF-positive cells between antrum and corpus was also reported [46].

According to a cell line research, *H. pylori* directly stimulated MIF secretion from monocytes, resulting in gastric cell proliferation through its *cag* PAI. The effect was blocked with anti-MIF antibody, suggesting the role of MIF as a mediator of *H. pylori*'s tumorigenic effect [47–51]. A clinical study showed a progressive increase of epithelial and serum MIF in *H. pylori*-induced gastritis, intestinal metaplasia, and GC. It suggested the potential role of MIF as a biomarker of GC [18]. A significant relationship between *H. pylori* and MIF is further supported by the report that the eradication of *H. pylori* reduced MIF in the patients [49]. A clinical study showed a possibility of MIF as a biomarker for GC. Serum MIF level in the patients had a diagnostic value better than carcinoembryonic antigen (CEA) and even correlated with the 5-year survival when combined with CEA [31]. MIF was also upregulated in the acute gastric ulcer rat model [52]. MIF, released upon *H. pylori* infection, induced phosphorylation of epidermal growth factor receptor (EGFR) [40]. However, the relationship between *H. pylori* and MIF needs to be interpreted carefully. A report found that in human gastric epithelial cells, MIF expression and secretion did not directly increase after *H. pylori* infection, although IL-8 expression and secretion were upregulated [51].

Human duodenal bulb mucosal samples of duodenal ulcer patients showed that MIF protein and mRNA expression were statistically different before and after eradication treatment. It suggests that the presence of *H. pylori* is closely related to the expression of MIF in the duodenal mucosa and the development of duodenal ulcer [53].

Gastric injury, pro-inflammatory factors, and oxidative stress in *H. pylori* infection produce asymmetric dimethylarginine (ADMA), which inhibits the activity of nitric oxide synthase (NOS). An animal study showed a significant increase in MIF expression, ADMA levels, and a significant positive correlation between MIF expression and ADMA levels in *H. pylori* infection [54].

25.5 Potential for Future Studies

Previous studies showed the clues for the role of MIF in gastric tumorigenesis. However, the exact mechanism remains elusive. MIF could potentially be an interesting topic, especially in relation to *H. pylori*. Here are some plausible topics for future research. First, it is interesting that CD74 makes up the receptor for MIF, and it also serves as the receptor for *H. pylori* at the same time [55–57]. This protein molecule could be a target to prevent and treat GC. It is also well known that MIF helps microangiogenesis. To investigate whether *H. pylori* is involved in this specific process could be also meaningful [58].

Its role in IL-6/JAK2/STAT3 pathway could be another target of research, especially with Th17 (IL-17) as a positive regulator through Akt [38, 59]. According to a recent study on nasal polyp, the inhibition of MIF enhanced the suppressive effect of dexamethasone on IL-6 [37]. It suggested that MIF inhibitor could be a novel therapy for the disease. The positive relationship of IL-6 to MIF might be another plausible topic in GC research.

In addition, MIF with autophagy can be an interesting object. In the human hepatoma cell line, MIF was reported to induce autophagy through reactive oxygen species generation. On the contrary, MIF was found to inhibit autophagy in breast cancer. Their interaction in GC, especially through *H. pylori*, is not yet clear [60–63].

25.6 Conclusions

MIF has been suggested to be involved in tumorigenesis of various types of cancer. Its positive interaction with *H. pylori* could be an attractive research topic in GC.

References

1. Li J, Mo HY, Xiong G, Zhang L, He J, Huang ZF, et al. Tumor microenvironment macrophage inhibitory factor directs the accumulation of interleukin-17-producing tumor-infiltrating lymphocytes and predicts favorable survival in nasopharyngeal carcinoma patients. J Biol Chem. 2012;287:35484–95.
2. Ren Y, Tsui HT, Poon RT, Ng IO, Li Z, Chen Y, et al. Macrophage migration inhibitory factor: roles in regulating tumor cell migration and expression of angiogenic factors in hepatocellular carcinoma. Int J Cancer. 2003;107:22–9.
3. Conroy H, Mawhinney L, Donnelly SC. Inflammation and cancer: macrophage migration inhibitory factor (MIF) – the potential missing link. QJM. 2010;103:831–6.
4. Xu X, Wang B, Ye C, Yao C, Lin Y, Huang X, et al. Overexpression of macrophage migration inhibitory factor induces angiogenesis in human breast cancer. Cancer Lett. 2008;261:147–57.
5. Bucala R, Donnelly SC. Macrophage migration inhibitory factor: a probable link between inflammation and cancer. Immunity. 2007;26:281–5.
6. Leng L, Metz CN, Fang Y, Xu J, Donnelly S, Baugh J, et al. MIF signal transduction initiated by binding to CD74. J Exp Med. 2003;197:1467–76.
7. Gore Y, Starlets D, Maharshak N, Becker-Herman S, Kaneyuki U, Leng L, et al. Macrophage migration inhibitory factor induces B cell survival by activation of a CD74-CD44 receptor complex. J Biol Chem. 2008;283:2784–92.
8. Shi X, Leng L, Wang T, Wang W, Du X, Li J, et al. CD44 is the signaling component of the macrophage migration inhibitory factor-CD74 receptor complex. Immunity. 2006;25:595–606.
9. Hudson JD, Shoaibi MA, Maestro R, Carnero A, Hannon GJ, Beach DH. A proinflammatory cytokine inhibits p53 tumor suppressor activity. J Exp Med. 1999;190:1375–82.
10. Meyer-Siegler K, Hudson PB. Enhanced expression of macrophage migration inhibitory factor in prostatic adenocarcinoma metastases. Urology. 1996;48:448–52.
11. Bando H, Matsumoto G, Bando M, Muta M, Ogawa T, Funata N, et al. Expression of macrophage migration inhibitory factor in human breast cancer: association with nodal spread. Jpn J Cancer Res. 2002;93:389–96.
12. White ES, Flaherty KR, Carskadon S, Brant A, Iannettoni MD, Yee J, et al. Macrophage migration inhibitory factor and CXC chemokine expression in non-small cell lung cancer: role in angiogenesis and prognosis. Clin Cancer Res. 2003;9:853–60.
13. Agarwal R, Whang DH, Alvero AB, Visintin I, Lai Y, Segal EA, et al. Macrophage migration inhibitory factor expression in ovarian cancer. Am J Obstet Gynecol. 2007;196(348):e1–5.
14. Han Y, Zhang C. Macrophage migration inhibitory factor plays a pivotal role in hepatocellular carcinoma and may be a noninvasive imaging target. Med Hypotheses. 2010;75:530–2.
15. Ren Y, Law S, Huang X, Lee PY, Bacher M, Srivastava G, et al. Macrophage migration inhibitory factor stimulates angiogenic factor expression and correlates with differentiation and lymph node status in patients with esophageal squamous cell carcinoma. Ann Surg. 2005;242:55–63.
16. Legendre H, Decaestecker C, Nagy N, Hendlisz A, Schuring MP, Salmon I, et al. Prognostic values of galectin-3 and the macrophage migration inhibitory factor (MIF) in human colorectal cancers. Mod Pathol. 2003;16:491–504.
17. Nabizadeh Marvast M, Sima HR, Ghaffarzadehgan K, Taghizadeh Kermani A, Norouzi N. Clinicopathological significance of macrophage migration inhibitory factor and its relation with p53 in gastric cancer. J Gastrointest Cancer. 2011;42:5–10.
18. He XX, Yang J, Ding YW, Liu W, Shen QY, Xia HH. Increased epithelial and serum expression of macrophage migration inhibitory factor (MIF) in gastric cancer: potential role of MIF in gastric carcinogenesis. Gut. 2006;55:797–802.
19. Li GQ, Xie J, Lei XY, Zhang L. Macrophage migration inhibitory factor regulates proliferation of gastric cancer cells via the PI3K/Akt pathway. World J Gastroenterol. 2009;15:5541–8.
20. White ES, Strom SR, Wys NL, Arenberg DA. Non-small cell lung cancer cells induce monocytes to increase expression of angiogenic activity. J Immunol. 2001;166:7549–55.
21. Wu MY, Fu J, Xu J, O'Malley BW, Wu RC. Steroid receptor coactivator 3 regulates autophagy in breast cancer cells through macrophage migration inhibitory factor. Cell Res. 2012;22:1003–21.

22. Yao K, Shida S, Selvakumaran M, Zimmerman R, Simon E, Schick J, et al. Macrophage migration inhibitory factor is a determinant of hypoxia-induced apoptosis in colon cancer cell lines. Clin Cancer Res. 2005;11:7264–72.
23. He XX, Chen K, Yang J, Li XY, Gan HY, Liu CY, et al. Macrophage migration inhibitory factor promotes colorectal cancer. Mol Med. 2009;15:1–10.
24. Muramaki M, Miyake H, Yamada Y, Hara I. Clinical utility of serum macrophage migration inhibitory factor in men with prostate cancer as a novel biomarker of detection and disease progression. Oncol Rep. 2006;15:253–7.
25. Michael A, Stephan C, Kristiansen G, Burckhardt M, Loening SA, Schnorr D, et al. Diagnostic validity of macrophage migration inhibitory factor in serum of patients with prostate cancer: a re-evaluation. Prostate. 2005;62:34–9.
26. Tang WM, Gou X, Liu QX. Expression of macrophage migration inhibition factor (MIF) in serum of patients with prostate cancer and its clinical significance. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2011;27:97–8.
27. Meyer-Siegler KL, Iczkowski KA, Vera PL. Further evidence for increased macrophage migration inhibitory factor expression in prostate cancer. BMC Cancer. 2005;5:73.
28. Meyer-Siegler KL, Iczkowski KA, Leng L, Bucala R, Vera PL. Inhibition of macrophage migration inhibitory factor or its receptor (CD74) attenuates growth and invasion of DU-145 prostate cancer cells. J Immunol. 2006;177:8730–9.
29. Krockenberger M, Kranke P, Häusler S, Engel JB, Horn E, Nürnberger K, et al. Macrophage migration-inhibitory factor levels in serum of patients with ovarian cancer correlates with poor prognosis. Anticancer Res. 2012;32:5233–8.
30. Tas F, Karabulut S, Serilmez M, Ciftci R, Duranyildiz D. Serum levels of macrophage migration-inhibitory factor (MIF) have diagnostic, predictive and prognostic roles in epithelial ovarian cancer patients. Tumour Biol. 2014;35:3327–31.
31. Xia HH, Yang Y, Chu KM, Gu Q, Zhang YY, He H, et al. Serum macrophage migration-inhibitory factor as a diagnostic and prognostic biomarker for gastric cancer. Cancer. 2009;115:5441–9.
32. Zheng YX, Yang M, Rong TT, Yuan XL, Ma YH, Wang ZH, et al. CD74 and macrophage migration inhibitory factor as therapeutic targets in gastric cancer. World J Gastroenterol. 2012;18:2253–61.
33. Camlica H, Duranyildiz D, Oguz H, Oral EN, Yasasever V. The diagnostic value of macrophage migration inhibitory factor (MIF) in gastric cancer. Pathol Oncol Res. 2008;14:79–83.
34. Mohri Y, Mohri T, Wei W, Qi YJ, Martin A, Miki C, et al. Identification of macrophage migration inhibitory factor and human neutrophil peptides 1–3 as potential biomarkers for gastric cancer. Br J Cancer. 2009;101:295–302.
35. Lee DH, Yang Y, Lee SJ, Kim KY, Koo TH, Shin SM, et al. Macrophage inhibitory cytokine-1 induces the invasiveness of gastric cancer cells by up-regulating the urokinase-type plasminogen activator system. Cancer Res. 2003;63:4648–55.
36. Shimwell NJ, Ward DG, Mohri Y, Mohri T, Pallan L, Teng M, et al. Macrophage migration inhibitory factor and DJ-1 in gastric cancer: differences between high-incidence and low-incidence areas. Br J Cancer. 2012;107:1595–601.
37. Stathas T, Athanassiou SD, Drakouli S, Giannopoulou E, Mastronikolis NS, Naxakis S, et al. MIF attenuates the suppressive effect of dexamethasone on IL-6 production by nasal polyp. Eur Rev Med Pharmacol Sci. 2013;17:1455–66.
38. Hsu HS, Lin JH, Hsu TW, Su K, Wang CW, Yang KY, et al. Mesenchymal stem cells enhance lung cancer initiation through activation of IL-6/JAK2/STAT3 pathway. Lung Cancer. 2012;75:167–77.
39. Petrenko O, Fingerle-Rowson G, Peng T, Mitchell RA, Metz CN. Macrophage migration inhibitory factor deficiency is associated with altered cell growth and reduced susceptibility to Ras-mediated transformation. J Biol Chem. 2003;278:11078–85.
40. Beswick EJ, Reyes VE. Macrophage migration inhibitory factor and interleukin-8 produced by gastric epithelial cells during *Helicobacter pylori* exposure induce expression and activation of the epidermal growth factor receptor. Infect Immun. 2008;76:3233–40.
41. Cooke G, Armstrong ME, Donnelly SC. Macrophage migration inhibitory factor (MIF), enzymatic activity and the inflammatory response. Biofactors. 2009;35:165–8.
42. Ohta S, Misawa A, Fukaya R, Inoue S, Kanemura Y, Okano H, et al. Macrophage migration inhibitory factor (MIF) promotes cell survival and proliferation of neural stem/progenitor cells. J Cell Sci. 2012;125(Pt 13):3210–20.
43. Fingerle-Rowson G, Petrenko O, Metz CN, Forsthuber TG, Mitchell R, Huss R, et al. The p53-dependent effects of macrophage migration inhibitory factor revealed by gene targeting. Proc Natl Acad Sci U S A. 2003;100:9354–9.
44. Talos F, Mena P, Fingerle-Rowson G, Moll U, Petrenko O. MIF loss impairs Myc-induced lymphomagenesis. Cell Death Differ. 2005;12:1319–28.
45. Chesney J, Metz C, Bacher M, Peng T, Meinhardt A, Bucala R. An essential role for macrophage migration inhibitory factor (MIF) in angiogenesis and the growth of a murine lymphoma. Mol Med. 1999;5:181–91.
46. Xia HH, Lam SK, Huang XR, Wong WM, Leung SY, Yuen ST, et al. *Helicobacter pylori* infection is associated with increased expression of macrophage migratory inhibitory factor – by epithelial cells, T cells, and macrophages—in gastric mucosa. J Infect Dis. 2004;190:293–302.
47. Xia HH, Lam SK, Chan AO, Lin MC, Kung HF, Ogura K, et al. Macrophage migration inhibitory factor stimulated by *Helicobacter pylori* increases proliferation of gastric epithelial cells. World J Gastroenterol. 2005;11:1946–50.

48. Handa O, Naito Y, Yoshikawa T. CagA protein of *Helicobacter pylori*: a hijacker of gastric epithelial cell signaling. Biochem Pharmacol. 2007;73:1697–702.
49. Kebapcilar L, Bilgir O, Cetinkaya E, Akyol M, Bilgir F, Bozkaya G. The effect of *Helicobacter pylori* eradication on macrophage migration inhibitory factor, C-reactive protein and fetuin-a levels. Clinics. 2010;65:799–802.
50. Li H, Zang J, Wang P, Dai L, Zhang J, Wang K. Gastric cancer susceptibility in gastric cancer relatives: attributable risks of macrophage migration inhibitory factor promoter polymorphism and *Helicobacter pylori*. Cytokine. 2012;60:346–51.
51. Lebiedz P, Heidemann J, Lugering A, Riedel S, Herbst H, Domschke W, et al. Gastric epithelial expression of macrophage migration inhibitory factor is not altered by *Helicobacter pylori* infection in humans. Helicobacter. 2006;11:258–65.
52. Huang XR, Chun Hui CW, Chen YX, Wong BC, Fung PC, Metz C, et al. Macrophage migration inhibitory factor is an important mediator in the pathogenesis of gastric inflammation in rats. Gastroenterology. 2001;121:619–30.
53. Yu XH, Zhang Q, Yang XP, Yang W, Dai F, Qian Z, et al. Expression of macrophage migration-inhibitory factor in duodenal ulcer and its relation to *Helicobacter pylori* infection. Genet Mol Res. 2015;14:13860–7.
54. Yulizal OK, Lelo A, Ilyas S, Kusumawati RL. Correlation of macrophage migration inhibitory factor (MIF) expression and asymmetric dimethylarginine (ADMA) levels in *Helicobacter pylori* infection. ICBBT 2020: 2020 12th International Conference on Bioinformatics and Biomedical Technology; May 22–24, 2020; Xi'an, China. New York: Association for Computing Machinery; 2020.
55. Beswick EJ, Pinchuk IV, Minch K, Suarez G, Sierra JC, Yamaoka Y, et al. The *Helicobacter pylori* urease B subunit binds to CD74 on gastric epithelial cells and induces NF-kappaB activation and interleukin-8 production. Infect Immun. 2006;74:1148–55.
56. McClelland M, Zhao L, Carskadon S, Arenberg D. Expression of CD74, the receptor for macrophage migration inhibitory factor, in non-small cell lung cancer. Am J Pathol. 2009;174:638–46.
57. Beswick EJ, Reyes VE. CD74 in antigen presentation, inflammation, and cancers of the gastrointestinal tract. World J Gastroenterol. 2009;15:2855–61.
58. Osinsky S, Bubnovskaya L, Ganusevich I, Kovelskaya A, Gumenyuk L, Olijnichenko G, et al. Hypoxia, tumour-associated macrophages, microvessel density, VEGF and matrix metalloproteinases in human gastric cancer: interaction and impact on survival. Clin Transl Oncol. 2011;13:133–8.
59. Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H. IL-17 can promote tumor growth through an IL-6-Stat3 signaling pathway. J Exp Med. 2009;206:1457–64.
60. Kang MR, Kim MS, Oh JE, Kim YR, Song SY, Kim SS, et al. Frameshift mutations of autophagy-related genes ATG2B, ATG5, ATG9B and ATG12 in gastric and colorectal cancers with microsatellite instability. J Pathol. 2009;217:702–6.
61. Kim MS, Jeong EG, Ahn CH, Kim SS, Lee SH, Yoo NJ. Frameshift mutation of UVRAG, an autophagy-related gene, in gastric carcinomas with microsatellite instability. Hum Pathol. 2008;39:1059–63.
62. Chuang YC, Su WH, Lei HY, Lin YS, Liu HS, Chang CP, et al. Macrophage migration inhibitory factor induces autophagy via reactive oxygen species generation. PLoS One. 2012;7:e37613.
63. Huang XW, Luo RH, Zhao Q, Shen ZZ, Huang LL, An XY, et al. *Helicobacter pylori* induces mitochondrial DNA mutation and reactive oxygen species level in AGS cells. Int J Med Sci. 2011;8:56–67.

Gastric Cancer: Epithelial-Mesenchymal Transition

26

Nayoung Kim, Yoon Jin Choi, and Hyeon Jang

Abstract

An epithelial-mesenchymal transition (EMT) induces loss of epithelial cell polarity and change of cell phenotype from typical cubical shape into fibroblast-like shape. Since the ability to migrate is obtained as a result of EMT, this has been an essential mechanism for cancer invasion and metastatic spreads. During EMT process, E-cadherin, a typical epithelial cell marker, is decreased, and hallmarks of mesenchymal cells are increased. Most studies about EMT in oncologic field have been focused on the role of EMT hallmarks as a prognostic marker. However, recent evidence has shown that EMT plays an important role in tumorigenesis and progression. In addition, EMT has taken center stage as the convergence point between inflammation and cancer.

This reviewed the general concept of EMT, a brief summary of the regulators and signal pathways involved in EMT, and the association between EMT and cancer including both carcinogenesis and progression. In terms of *Helicobacter pylori* (*H. pylori*)-associated gastric cancer (GC), EMT plays a major role in the development of tumor microenvironment and cancer stem cells (CSC). Tumor necrosis factor-α-inducing protein (Tipα) was suggested to promote EMT and CSC-like properties in GC cells through activation of Wnt/β-catenin signaling pathway, thereby accelerating the progression of GC.

Keywords

Helicobacter pylori · Epithelial-mesenchymal transition · Cancer stem cell · Gastric cancer

26.1 Introduction

Epithelial-mesenchymal transition (EMT) is a process in which epithelial cells become mesenchymal stem cells. It is an example of cell plasticity, in which one cell could be changed to another type of cell. Epithelial cells have cell junctions: tight junctions, adherent junctions, desmosomes, hemidesmosomes, and gap junctions, by which epithelial cells are tightly packed. As a result, epithelial cells exhibit apical-basal polarity and have a role in protection, secretion, selective

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absorption, and cell–cell transduction [1]. In contrast, mesenchymal cells do not have polarity and thus have cellular mobility. Also, it can be differentiated into functional cells: osteoblast, chondrocyte, and adipocyte mainly due to microenvironment [2, 3]. For instance, the cuboidal epithelial cell phenotype loses cellular polarity and thus changes into the types of fibroblast. Furthermore, expressions of epithelial markers are decreased with an increase of mesenchymal markers.

E-cadherin, distributed in/out of cell membrane, is a typical epithelial cell marker and is located in an adhering junction of epithelial cells and has several roles for cytoskeleton and maintenance of continuous sheet. Cadherin switch which occurs in the early stage of EMT induces E-cadherin loss and increment of vimentin and N-cadherin. It results in phenotypical and functional change for weakening of cell–cell adhesion and enhancing cellular mobility [4]. Cellular microenvironment and tissue specificity can divide EMT into three types: embryogenesis, tissue regeneration and organ fibrosis, and carcinogenesis and metastasis [5].

This chapter briefly explains the overall regulation and inducers of EMT in specific type 3 EMT. The role of EMT in tissue invasiveness, metastasis, and gastric carcinogenesis is described as well. Then, the importance of *Helicobacter pylori* (*H. pylori*) infection as EMT inducers is shown. In the next, the role of EMT in the *H. pylori*-associated gastric cancer (GC) has been introduced. EMT, tumor microenvironment and cancer stem-like cells (CSC)-like properties are closely related through several mechanisms, thereby accelerating the progression of GC.

26.2 Three Types of EMT

Two meetings in 2007 and 2008 had distinguished EMT into three types in tissue and function specific [6]. Type 1 EMT is involved with embryogenesis and appropriate organ development. Type 2 EMT is engaged in tissue regeneration and organ fibrosis [7]. Type 3 EMT is associated with cancer progression, tissue invasiveness, metastasis, recurrence, and resistance. Also, metastatic cancer cell can get mesenchymal phenotype through type 3 EMT [8, 9]. While the distinct functions are apparent in different tissues, specific signals driven in these EMTs are similarly induced by genetic and biochemical factors in cells.

26.2.1 Type 1: Embryogenesis

Type 1 EMT regulates the cellular growth of mesenchymal phenotype cell which associates implantation, embryogenesis, and organ development. Especially, secondary epithelium which can induce mesenchymal-epithelial transition (MET) would arise from this type 1 EMT [9–11] (Fig. 26.1a).

26.2.2 Type 2: Tissue Regeneration and Organ Fibrosis

Type 2 EMT is engaged in tissue regeneration after wound and inflammatory tissue degradation. Fibroblast and inflammatory cells are involved in tissue regeneration, and after wound healing, type 2 EMT would be ceased, but chronic inflammation caused by recurrence of infection, autoimmune, and allergy would induce excessive tissue regeneration and result in tissue fibrosis [12–14] (Fig. 26.1b).

26.2.3 Type 3: Invasiveness and Metastasis of Cancer

The relationship between chronic inflammation and carcinogenesis indicates that EMT would increase the risk of carcinogenesis [13]. Mutation of oncogene and tumor suppressor genes induces serial changes of EMT signaling, and this distinguishes distinct physiological phenotypes from type 1 and 2 EMTs. After the initiation of type 3 EMT, tumor cells can invade into the circulatory system, metastasize in other organs, and finally promote newly formed tumor (Fig. 26.1c).

As EMT progresses, tumor cell loses epithelial cell markers, E-cadherin, and cytokeratin and

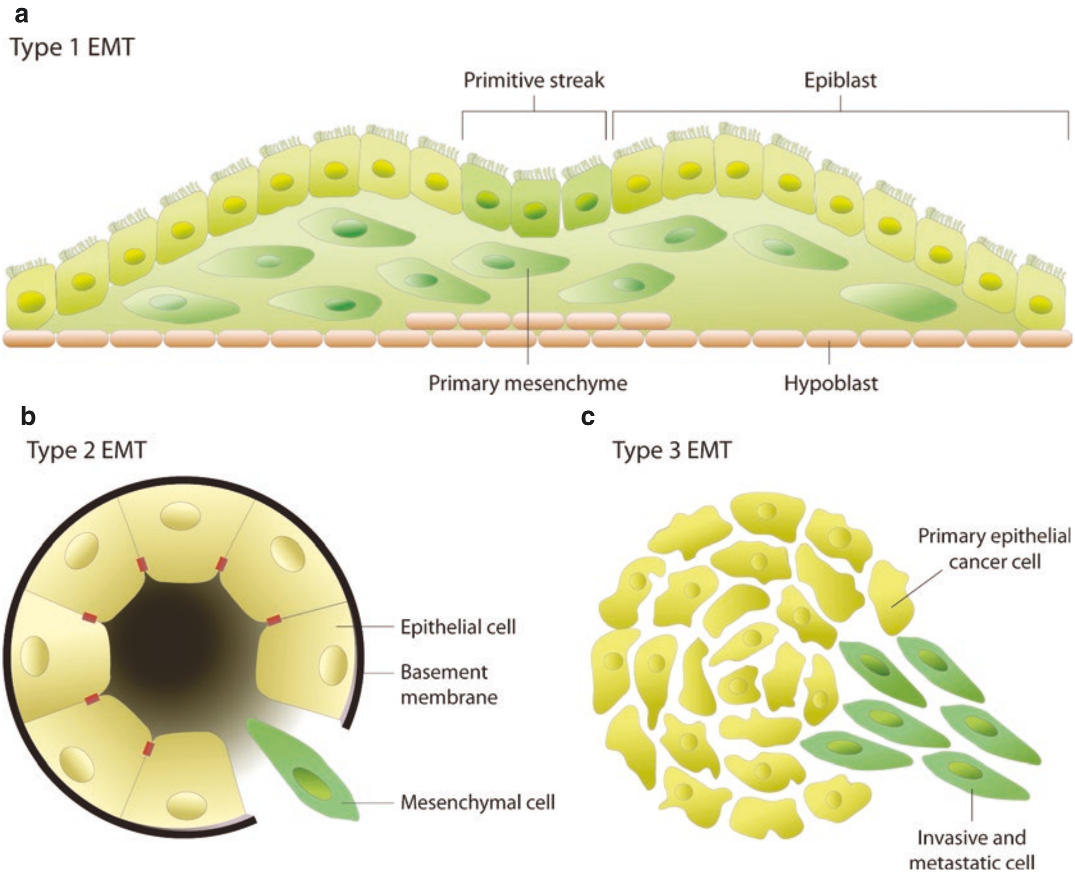
a
Type 1 EMT
Primitive streak
Epiblast
Primary mesenchyme
Hypoblast
b
Type 2 EMT
Epithelial cell
Basement membrane
Mesenchymal cell
c
Type 3 EMT
Primary epithelial cancer cell
Invasive and metastatic cell

Fig. 26.1 Three types of EMT. (**a**) Type 1 EMT associated with embryogenesis and organ development. (**b**) Type 2 EMT associated with tissue regeneration and organ fibrosis. (**c**) Type 3 EMT associated with cancer progression and metastasis. *EMT* epithelial-mesenchymal transition (adapted from Kalluri et al. [9])

acquire mesenchymal cell markers, α-smooth muscle actin (α-SMA), N-cadherin, and vimentin [9] (Fig. 26.2). This newly changed tumor cells are observed in border between central region of cancer and surrounding tissues. Then, the tumor cell finally establishes micrometastasis after migration [15].

Many researches try to disclose the gene regulation and biochemical mechanism that cause proliferation of tumor cells, and most of those studies recommend that activation of EMT could be a reason for the malignant alteration in epithelial tumor cells [16]. Moreover, metastasized tumor cells do not have mesenchymal cell phenotype in the secondary site after EMT progression, but they have the epithelial tumor cell phenotype which is shown in the primary site. Thus, in addition to the metastasis promotion ability of EMT, it is needed to comprehend that MET would induce loss of mesenchymal cell phenotypes after metastasis [17] (Fig. 26.3).

It is implicated that EMT progression in metastatic cancer cell would associate with cellular microenvironment, but further studies are needed [18]. Also, the mechanisms for mesenchymal cell phenotype acquisition of non-epithelial cell are not yet clear, but EMT is regarded as a principal mechanism for metastatic cancer progression as well [19].

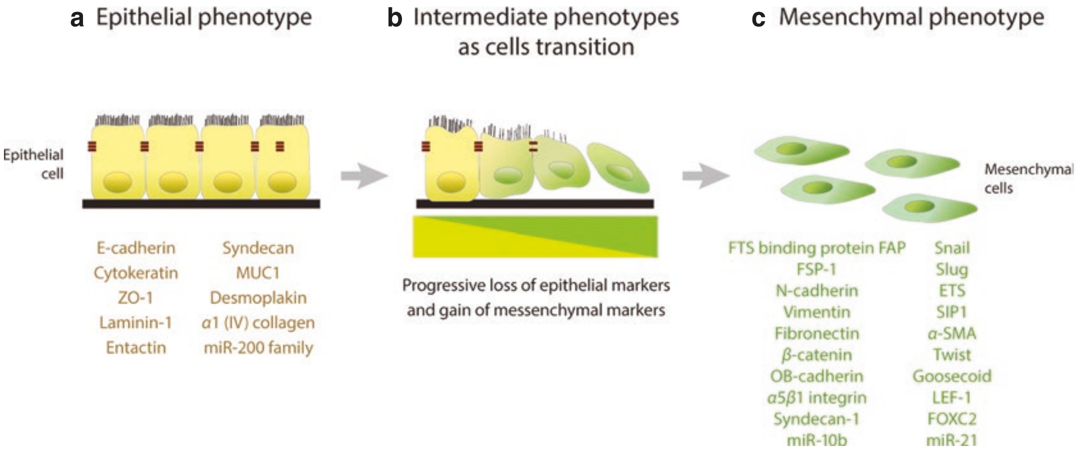
a Epithelial phenotype
b Intermediate phenotypes as cells transition
c Mesenchymal phenotype
Epithelial cell
Mesenchymal cells
E-cadherin
Cytokeratin
ZO-1
Laminin-1
Entactin
Syndecan
MUC1
Desmoplakin
α1 (IV) collagen
miR-200 family
Progressive loss of epithelial markers and gain of messenchymal markers
FTS binding protein FAP
FSP-1
N-cadherin
Vimentin
Fibronectin
β-catenin
OB-cadherin
α5β1 integrin
Syndecan-1
miR-10b
Snail
Slug
ETS
SIP1
α-SMA
Twist
Goosecoid
LEF-1
FOXC2
miR-21

Fig. 26.2 Change of EMT marker expression. (**a**) Epithelial cell markers. (**b**) Epithelial cell markers decreased and mesenchymal cell markers increased in EMT. (**c**) Mesenchymal cell markers. *ZO-1* zona occludens 1, *MUC1* mucin 1, *SIP1* Smad-interacting protein 1, *α-SMA* α-smooth muscle actin, *FOXC2* forkhead box C2 (adapted from Kalluri et al. [9])

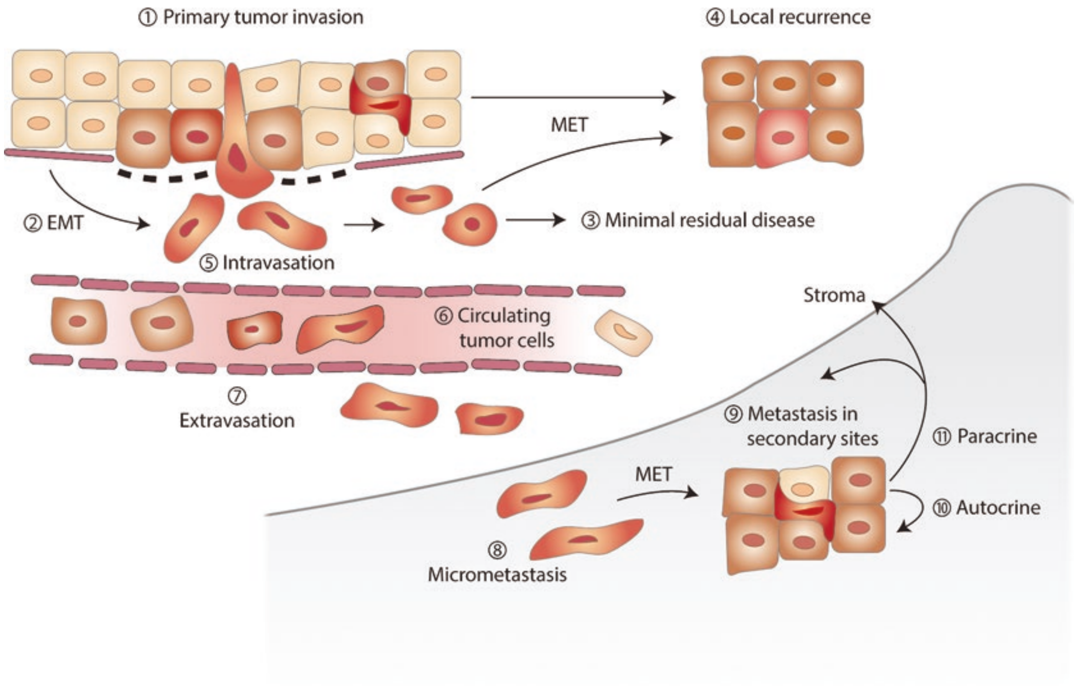
① Primary tumor invasion
④ Local recurrence
MET
② EMT
③ Minimal residual disease
⑤ Intravasation
Stroma
⑥ Circulating tumor cells
⑦ Extravasation
⑨ Metastasis in secondary sites
⑪ Paracrine
MET
⑩ Autocrine
⑧ Micrometastasis

Fig. 26.3 Metastasis procedure of EMT and/or MET. (*1*) Primary tumor invasion. (*2*) Tissue invasion after EMT. (*3*) Minimal residual disease occurs as well. (*4*) MET occurs in residual mesenchymal cell. Local recurrence arises. (*5*) Mesenchymal cell easily achieves intravasation. (*6*) Circulating tumor cells can exist as epithelial cell and/or mesenchymal phenotype cell. (*7*) Mesenchymal cell easily achieves extravasation. (*8*) Tumor cell can exist as micrometastasis. (*9*) After the incubation period, metastasis in secondary sites and MET occur. (*10*) Autocrine and (*11*) paracrine would promote metastatic tumor cell growth (adapted from Thompson and Haviv [17])

26.3 Major Signal Pathways of EMT

It is impossible to understand the whole EMT signaling of tumor cells, but the most persuasive hypothesis suggests that genetic and epigenetic signals that occurred in the early stage of carcinogenesis would change tumor cells to be susceptible to the EMT. The inhibition of tumor cell senescence would associate with the early step of carcinogenesis in EMT progression [20, 21]. Various EMT-inducing signals, hepatocyte growth factor (HGF), epithelial growth factor (EGF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF)-β can promote EMT in basal site of tumor and activate several transcription factors: Snail, Slug, zinc finger E-box binding homeobox 1 (ZEB1), and Twist [8, 22–25] (Fig. 26.4). Activation for transcription factors needs mitogen-activated protein kinase (MAPK), phosphatidylinositol 3′ kinase (PI3K), serine/threonine kinase (Akt), glycogen synthase kinase-3β (GSK-3β), integrin-linked kinase (ILK), Smads, RhoA, Par6, β-catenin, and Ras signaling which interacts with signal transduction protein [26] (Fig. 26.5). Disruption of cell–cell adhesion or cell-basal adhesion by integrin also promotes EMT program activation [27–30].

TGF-β/Smad signaling, the central axis of type 3 EMT, and Wnt/β-catenin signaling and Notch signaling are introduced next.

26.3.1 TGF-β/Smad Signaling

TGF-β can inhibit unregulated proliferation of epithelial cells or induce carcinogenesis and metastasis in different environments. Also, TGF-β is the most well-known EMT inducer. It would activate EMT via two kinds of cellular signaling (Smad/non-Smad) [26, 31–33] (Fig. 26.5).

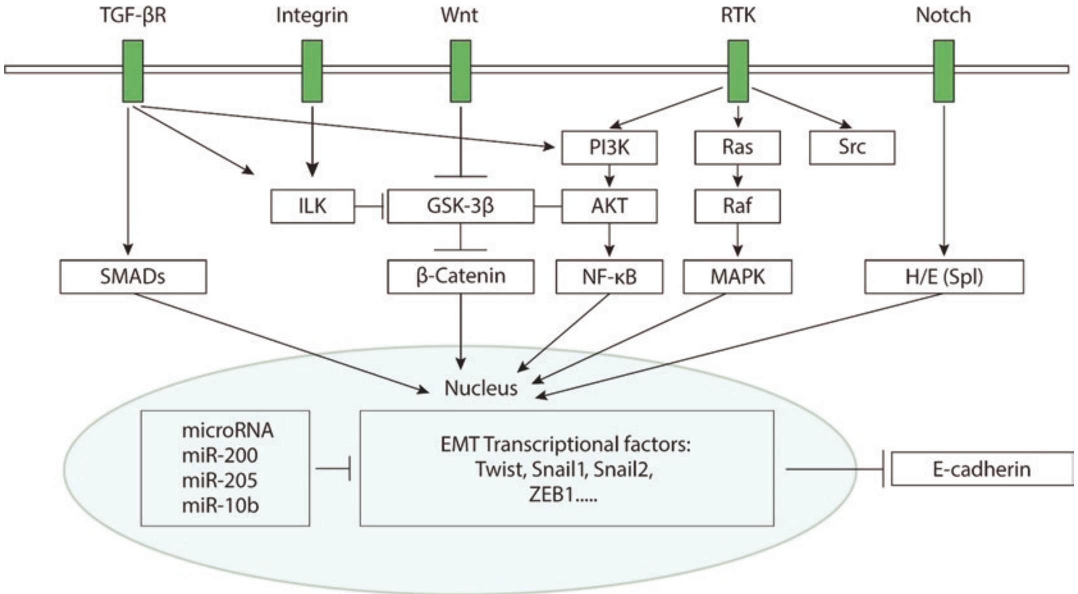

Fig. 26.4 Scheme of major EMT induction signal. TGF-β transduces through SMAD and PI3K/AKT. Wnt ligand inhibits β-catenin senescence. Accumulated β-catenin can enter into the nucleus and increase transcription factors Slug and Snail. The integrin signal induces hyperactivation of ILK, and this helps β-catenin invagination to the nucleus. RTK signal induces EMT through Ras-Raf-MAPK or PI3K/AKT. *AKT* serine/threonine kinase, *GSK-3β* glycogen synthase kinase-3β, *H/E* (*Spl*) Hairy and enhancer of split, *ILK* integrin-linked kinase, *MAPK* mitogen-activated protein kinase, *NF-κB* nuclear factor-κB, *PI3K* phosphatidylinositol 3′ kinase, *RTK* receptor tyrosine kinase, *TGF-βR* transforming growth factor-β receptor, *ZEB1* zinc finger E-box binding homeobox 1 (adapted from Iwatsuki et al. [8])

26.3.1.1 Smad Signaling

When TGF-β attaches to type I, type II activin receptor-like-kinase (ALK) receptor and intrinsic serine/threonine kinase are activated. Smad2 and Smad3 are phosphorylated and attach with Smad4 and achieve the invagination into the nucleus [26] (Fig. 26.5). After that, transcription factors such as Snail expression would regulate the expression of connective tissue growth factor, α-SMA, plasminogen activator inhibitor-1, and collagen 1A2 [34–37].

26.3.1.2 Non-Smad Signaling

Non-Smad signaling induces EMT with various cells and microenvironment-specific proteins. Activation of these signal transduction proteins

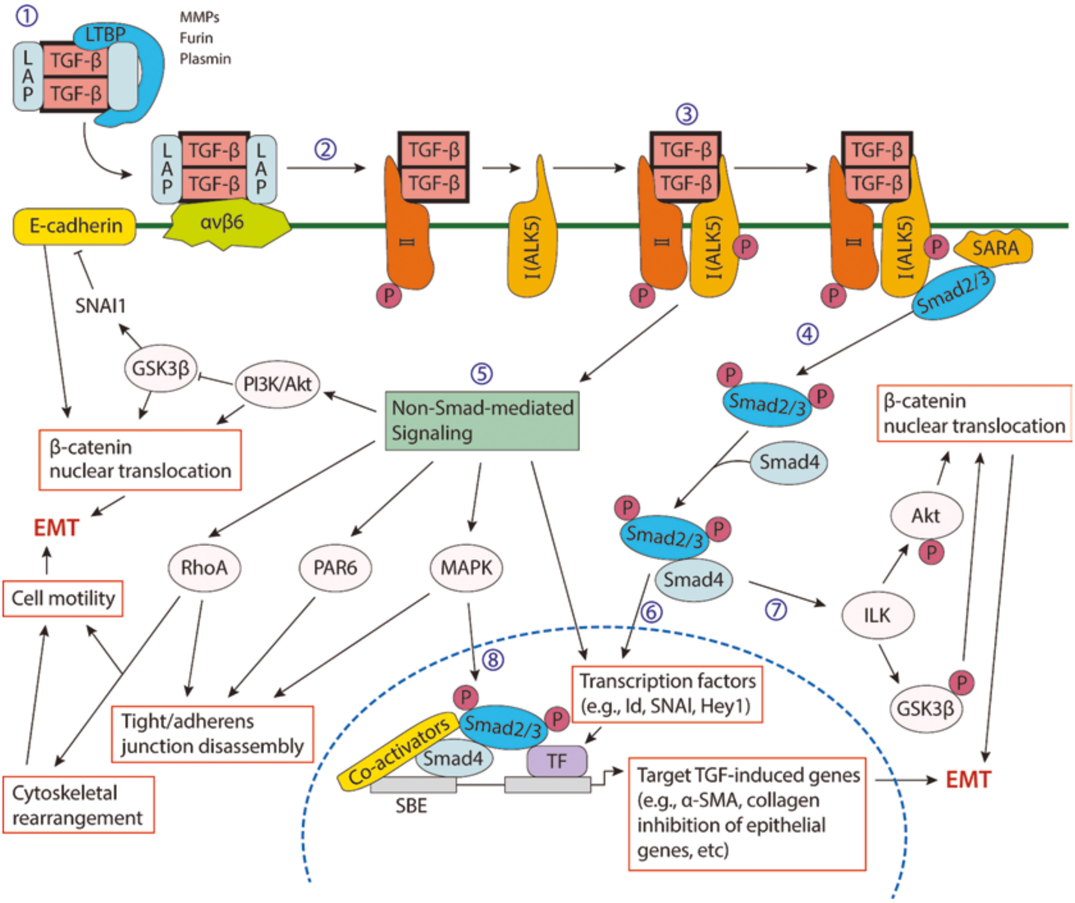
①
LTBP
LAP
TGF-β
TGF-β
MMPs
Furin
Plasmin
LAP
TGF-β
TGF-β
LAP
②
αvβ6
E-cadherin
TGF-β
TGF-β
II
P
I (ALK5)
③
TGF-β
TGF-β
II
I (ALK5)
P
P
TGF-β
TGF-β
II
I (ALK5)
P
P
SARA
Smad2/3
SNAI1
GSK3β
PI3K/Akt
④
⑤
Non-Smad-mediated Signaling
P
Smad2/3
P
Smad4
β-catenin nuclear translocation
β-catenin nuclear translocation
EMT
P
Smad2/3
P
Smad4
Akt
P
Cell motility
RhoA
PAR6
MAPK
⑥
⑦
ILK
⑧
Transcription factors (e.g., Id, SNAI, Hey1)
P
GSK3β
Tight/adherens junction disassembly
P
Smad2/3
P
Co-activators
Smad4
TF
SBE
Target TGF-induced genes (e.g., α-SMA, collagen inhibition of epithelial genes, etc)
EMT
Cytoskeletal rearrangement

Fig. 26.5 EMT induced by transforming growth factor (TGF)-β1 signaling. (*1*) TGF-β is released. (*2*) TGF-β dimers fuse with TGF-β receptors (e.g., ALK-5). (*3*) Activation of receptor heterodimer promotes, (*4*) Smad (Smad2/Smad3), and/or (*5*) non-Smad signaling. (*6*) Smad signaling activates Snail1, Snail2, Notch transcription factor, and TGF-β-induced target gene (e.g., α-smooth muscle actin, collagen, plasminogen activator inhibitor-1, connective tissue growth factor) and inhibits epithelial cell gene (e.g., E-cadherin). (*7*) Smad signaling also activates ILK, and this results in Akt/GSK3B activation. Finally, β-catenin can enter into the nucleus and induce further EMT program activation. (*5*) Non-Smad signaling induces activation of PI3K/Akt, RhoA, PAR6, and MAPK and disruption of tight junction/adhering junction and rearrangement of cytoskeleton and inhibits E-cadherin and promotes of β-catenin invagination into the nucleus. (*8*) Smad signaling and non-Smad signaling interact with MAPK. *SNAI1* snail family zinc finger 1, *LTBP* latent TGF-binding protein, *LAP* latency-associated peptide, *GSK3β* glycogen synthase kinase-3β, *ILK* integrin-linked kinase, *SARA* Smad anchor for receptor activation, *RhoA* Ras homolog gene family, member A, *PAR6* partitioning-defective 6, *Id* inhibitor of DNA binding 1, *Hey1* hairy/enhancer of split related to YRPW motif protein 1 (adapted from Willis and Borok [26])

is overlapped in EMT. RhoA and MAPK mediate EMT which is induced by TGF-β in animal epithelial cells [37–40]. At the same time, PI3K activation after Ras would inhibit cellular apoptosis that is induced by TGF-β [41–44]. COX-2 inhibits Smad signaling and causes hyperexpression of prostaglandin E2. Then, this protein finally enhances EMT signaling induced by TGF-β [45, 46].

26.3.2 Wnt/β-Catenin Signaling

When Wnt signal is activated and β-catenin is dephosphorylated, β-catenin in cell–cell adhesion would achieve invagination into the nucleus. Then, epithelial cells have mesenchymal cell phenotypes. Thus, the expression of β-catenin in the cytoplasm and inhibition of its activation is essential to keep the epithelial tumor cell phenotype [47]. Accumulation of β-catenin in the nucleus is related to loss of E-cadherin expression and is highly correlated with intravasation phenotype after EMT [48, 49].

26.3.3 Notch Signaling

Notch signaling has an important role in the birth of cancer stem cells (CSC). The hyperexpression and hyperactivation of Notch protein are reported in various solid cancers including GC. And Notch is associated with Snail, Slug, and TGF-β [49].

26.4 EMT and Gastric Cancer

H. pylori virulence factors such as cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) are considered being associated with the promotion of EMT in gastric cells, which consequently cause neoplasia and malignant transformation [50]. In addition, various EMT factors are involved in *H. pylori*-associated GC.

26.4.1 Association Between Gastric Cancer and EMT

Although the incidence of GC is definitely decreasing, GC is still the second most common cause of cancer-related death. Gastric adenocarcinoma accounts for 90% of GC, and this is divided into intestinal type and diffuse type according to Lauren classification. Even though there is a study that reported that different mechanisms underlie different Lauren types, downregulation of E-cadherin seems to be an essential component for EMT-related carcinogenesis. That is, (1) genetic abnormality, mutation, and deletion; (2) upregulation of E-cadherin suppressing transcription factor; and (3) epigenetic regulation [i.e., microRNA (miRNA) or hypermethylation of specific genes] result in a functional and morphological decline of E-cadherin [51]. In addition, amplification or mutation of receptors in other EMT signal pathways could contribute to a promotion of EMT leading to GC [52, 53].

26.4.1.1 CagA, Cytotoxin-Associated Gene Toxin

Among many virulence factors of *H. pylori*, *cag* pathogenicity island (*cag* PAI) probably plays a key role in carcinogenesis [54]. It encodes a type 4 secretion system (T4SS) and the CagA oncoprotein [55]. The T4SS forms a pilus that allows the injection of CagA into a cell, transforming its shape into the so-called hummingbird phenotype characterized by an elongated cell shape commonly observed in EMT [56]. Injection of the CagA into the cell via the T4SS induces signal transduction which is related to the nuclear factor κB (NF-κB) signaling pathway involving extracellular regulated kinases 1/2 (ERK-1/2) [57]. These kinases are involved in the conformational changes of the cytoskeleton, which might enhance the EMT process [58].

In addition, the apoptosis-stimulating protein of p53–2 (ASPP2), a host tumor suppressor and an important CagA target, has been known to contribute to the survival of *cagA*-positive *H. pylori* in the lumen of infected gastric organoids [59]. Mechanistically, the CagA-ASPP2 interaction was a key event that the promoted remodeling of the partitioning-defective polarity complex [59]. Blockade of *cagA*-positive *H. pylori* ASPP2 signaling by inhibitors of the epidermal growth factor receptor (EGFR) or by a CagA-binding ASPP2 peptide prevented the loss of cell polarity and decreased the survival of *H. pylori*-infected organoids [59]. These findings suggest that maintaining the host cell-polarity barrier would reduce the detrimental consequences of *H. pylori* infection [59].

26.4.1.2 VacA, Vacuolating Cytotoxin

H. pylori induces chronic inflammation of the gastric mucosa which is associated with high oxidative stress [60]. Recently, it was found that the downregulation of nuclear factor erythroid 2-related factor 2 (Nrf2), a transcriptional factor and major regulator of cellular antioxidant response to oxidative stress, contributes to EMT in *H. pylori*-infected cells in relation to VacA [60]. Nrf2 plays a critical role in maintaining cellular redox homeostasis by activating the transcription of numerous genes (heme oxygenase-1, *HO1*; NAD(P)H quinone dehydrogenase 1, *NQO1*; and both subunits forming glutathione GSH: glutamate-cysteine ligase modifier, *GCLM,* and glutamate-cysteine ligase catalytic, *GCLC*) involved in the antioxidant response [61]. This Nrf2 signaling pathway was found to be differentially regulated depending on the infection stage [60]. Rapidly and transiently activated, Nrf2 was downregulated 24 h postinfection in a VacA-dependent manner [60]. In the next, Nrf2 invalidation led to increased EMT, which was even exacerbated after *H. pylori* infection [60]. Finally, Nrf2 expression tended to decrease in human patients' gastric mucosa infected with *H. pylori* [60]. Taken together, these results suggest that Nrf2 downregulation upon *H. pylori* infection participates in EMT by way of VacA [60].

26.4.2 EMT Factors Related to Gastric Cancer

26.4.2.1 Regulation of E-Cadherin

Functional Loss Through CDH1 Mutation

CDH1 gene at human chromosome 16q22.1 encodes E-cadherin [51]. Mutation, deletion, and CpG hypermethylation of *CDH1* gene in GC have been reported [62]. Germ-line mutations of *CDH1* result in hereditary diffuse-type GC. This genetic mutation also leads to inactivation of other alleles located in patients with familial GC of diffuse type.

Repression for the Transcription of CDH1

Snail, Slug, Smad-interacting protein 1 (SIP1), and Twist act as repressors for the transcription of *CDH1*, and this leads to EMT [24]. It is reported that there was an inverse correlation between the abovementioned transcription factors and CDH1 in various types of cancer. These facts indicated that Snail, Slug, SIP1, or Twist was implicated in the EMT in GC through the transcriptional repression of the *CDH1* gene.

Snail: According to the study which evaluated the expression of Snail and E-cadherin in human GC mucosa, the downregulation of E-cadherin (up to 40%) and upregulation of Snail and N-cadherin were reported in diffuse type without changes in SIP1 expression [63]. Contrary to this, approximately 60% of patients with intestinal-type GC showed a decrease in E-cadherin expression and an increase in SIP1 without any change in expressions of Snail, Twist, or N-cadherin. These results suggested that the expression of Snail was associated with the suppression of E-cadherin in diffuse-type GC, while SIP1 inhibits E-cadherin expression. Different regulators may act according to the histological type of GC [64, 65]. In addition, *H. pylori* induced Snail expression through reactive oxygen species (ROS)-mediated activation of Erk and inactivation of GSK-3β in AGS cells [66]. There was another report regarding Snail and CagA-induced gastric carcinogenesis [67]. Casein kinase 2 (CK2) is a serine/threonine protein kinase that plays a major role in tumorigenesis through signaling pathways related to the EMT. Although CK2α protein expression remained unchanged during *H. pylori* infection, CK2α kinase activity increased in gastric epithelial cells (AGS and MKN74 cell lines) [67]. In addition, the CK2β protein level decreased in *H. pylori*-infected GC cells in CagA-dependent manner. For example, CagA induced CK2β degradation via human double minute 2 (HDM2, its murine equivalent is MDM2). Interestingly, this downregulation of

CK2β triggered the upregulation of Snail levels in GC cells. Taken together, CK2 could be a key mediator of the EMT in *H. pylori*-infected GC and could serve as a molecular target for GC treatment by way of Snail.

Slug: Transcription factor Slug is included in the Snail family. It represses the expression of E-cadherin as well. It is well known that Slug expression is considerably increased in intestinal type and diffuse type of GC. Also, other studies elucidated that Slug expression in GC tissue is higher than that of normal tissue about 70%, and it is statistically significant. Increased Slug expression is correlated with the loss of E-cadherin [63].

Twist: The expression of helix–loop–helix transcription factor, Twist, and N-cadherin is mainly found in diffuse-type GC. Repression of Twist in GC cell resulted in loss of N-cadherin expression and decrease of cell mobility. However, when N-cadherin expression increased, cell mobility would increase, and E-cadherin expression was repressed [68]. Thus, it was identified that Twist is a key factor for the induction of "cadherin switch" which showed the change of E-cadherin into N-cadherin, and it would increase cancer cell mobility [69]. The fibroblast-specific protein 1 and CXCL14 are highly expressed in fibroblast of GC tissue as Twist expression. Also, Twist mRNA expression is correlated with PDGF receptor-α and receptor-β. This means that high expression of Twist would increase EMT sensitivity of GC tissue, and it consequently promotes cancer cell development and metastasis [70].

26.4.2.2 Epigenetic Regulation of EMT via miRNA

Small RNA, miRNA, consists of 17–22 nucleotides, and it represses gene expression by irreversible attachment on mRNA in mRNA transcription. Studies that identified the role of miRNA in metastatic cancer also gave the information on EMT-regulating miRNAs [71] (Fig. 26.6 and Table 26.1). MiR-27 is mainly

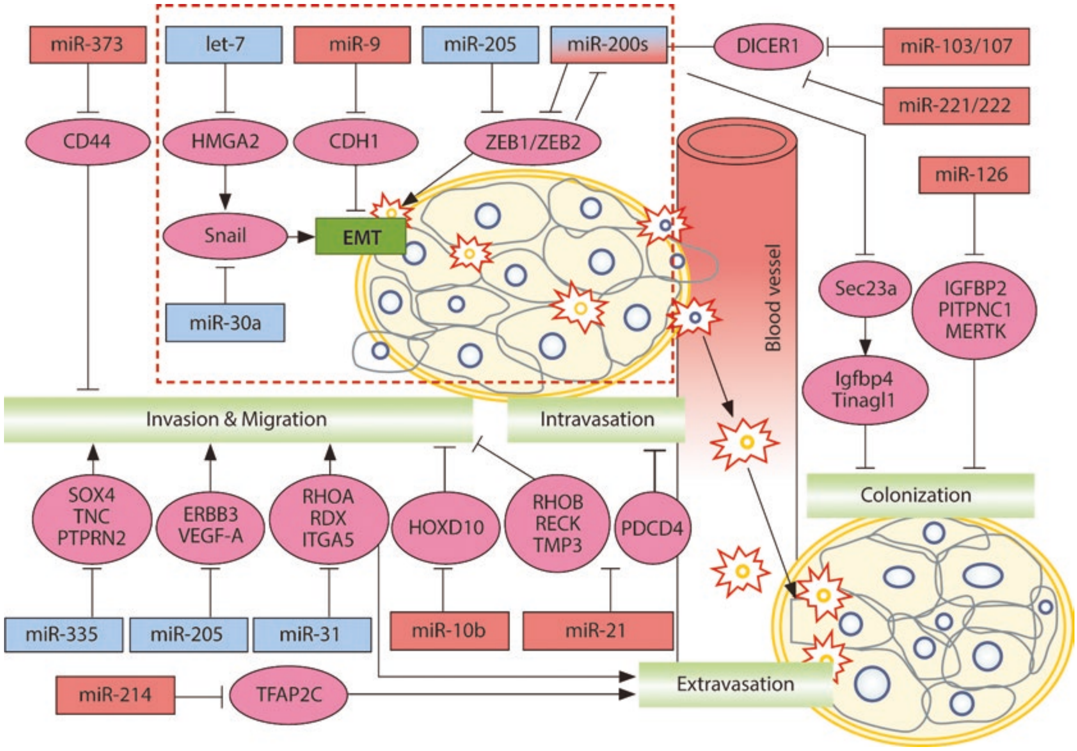
miR-373
let-7
miR-9
miR-205
miR-200s
DICER1
miR-103/107
miR-221/222
CD44
HMGA2
CDH1
ZEB1/ZEB2
miR-126
Snail
EMT
miR-30a
Blood vessel
Sec23a
IGFBP2
PITPNC1
MERTK
Igfbp4
Tinagl1
Invasion & Migration
Intravasation
Colonization
SOX4
TNC
PTPRN2
ERBB3
VEGF-A
RHOA
RDX
ITGA5
HOXD10
RHOB
RECK
TMP3
PDCD4
miR-335
miR-205
miR-31
miR-10b
miR-21
miR-214
TFAP2C
Extravasation

Fig. 26.6 miRNAs that regulate metastasis. Metastasis consists of multiple steps: epithelial-mesenchymal transition (*EMT*), local invasion, intravasation, extravasation, and colonization (as indicated by *green boxes*). MiRNAs and their target genes are indicated in *red/blue boxes* and *pink circles*, respectively. *Red box* metastasis-promoting miRNAs, *blue box* metastasis-suppressing miRNAs (adapted from Zhang et al. [71])

Table 26.1 miRNAs involved in epithelial-mesenchymal transition/mesenchymal-epithelial transition

miRNA	Effect on EMT	Target
miR-9	Promote	CDH1
miR-15b	Suppress	BMI1
miR-27	Promote	APC
miR-29a	Promote	TTP
miR-30a	Suppress	Snail
miR-103/107	Promote	DICER1
miR-155	Promote	RHOA
miR-194	Suppress	BMI1
miR-200 family	Suppress	ZEB1/ZEB2, Sec23a
miR-205	Suppress	ZEB1/ZEB2
miR-204	Suppress	TGFβR2, SNAIL2
miR-221/222	Promote	TRPS1, ESR1, DICER1
miR-661	Promote	StarD10, Nectin-1

Adapted from Zhang et al. [71]
EMT epithelial-mesenchymal transition, *miR* microRNA

studied for its relation to GC. It is usually upregulated in GC tissue compared to normal tissue [72]. MiR-27 would increase the mobility of AGS cells, the cancer cell line, and induce the expression of ZEB1, ZEB2, Slug, and vimentin, so miR-27 finally induced EMT [73]. Also, miR-27 expression would be increased from Wnt signal transduction, and it represses the activation of tumor suppressor gene adenomatous polyposis coli (APC) to promote metastasis of GC [73]. Other EMT-regulating miRNAs are included in miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) [71]. Because those miRNAs induce MET and repress EMT by regulating ZEB1 and ZEB2, the members of miR-200 family are known as EMT markers [71]. However, ZEB1 has a double negative feedback structure, so it can attach to the promoter of miR-200 [74]. Also, the upregulation of ZEB1/ZEB2 and reduction of miR-200 family resulted in the decrease in E-cadherin expression within metastatic cancer tissue which reduced E-cadherin expression [71]. In addition, miR-9 can repress mRNA of E-cadherin gene, *CDH1* [75], and miR-155 and can induce EMT by upregulating ROHA [76], but miR-204 is known as EMT repressor [77]. The most interesting part of gastric EMT is that *H. pylori* infection can repress miR-200 family and miR-204, but it increases miR-155 [78]. Therefore, it is implicated that *H. pylori* infection has an effect on the invasiveness and metastasis of GC with EMT-regulating miRNAs.

26.4.2.3 Other EMT Regulatory Factors

Vimentin

Vimentin is 58 kDa of intermediate filament protein, and it is not expressed in epithelial cells but in mesenchymal cells. Vimentin has a role in maintaining the cytoskeleton. When vimentin is expressed, it interacts with other intermediate filament proteins (i.e., actin) by inducing EMT and changing epithelial cells into migratory mesenchymal cells.

Bone Morphogenetic Protein (BMP)

Bone morphogenetic protein (BMP) is included in TGF-β family, and it is known for its role in the production of osteoinductive cytokine and the growth of bone and cartilage in embryonic development. BMP combines with the phosphorylation domain of serine/threonine BMP I/II receptor, and it phosphorylates Smad 1/5/8. Normally, phosphorylated Smad can intrude the nucleus and interact with transcription factors to activate cellular signal transduction. However, abnormal regulation of BMP would induce metastasis, invasiveness, and resistance against apoptosis in GC cells. Especially, it is supposed that BMP-2 and BMP-4 are considerably related to tumor metastasis. Because BMP-2 makes and activates BMP-2 receptors by autocrine process, BMP receptors which are expressed in the extracellular domain of cancer cells can bind with massive BMP-2 and promote EMT of GC cells [79–81].

Claudin

Claudin is one of the constructive proteins that compose the tight junction. It can repress tumor growth by RUNX3/Smad signaling and is usually hypermethylated in GC tissue. Claudin 1 expression is lowered in intestinal-type GC [82, 83].

Gastrokine

Gastrokine 1 is one of the gastric mucosal protective factors. Normally, it acts as a repressor against GC. When the gastric cell lines were transformed to be overexpressed with gastrokine I, it repressed migration, metastasis of GC cells, and expression of reactive oxygen species (ROS) [84]. In addition, when gastrokine 1 is upregulated, it represses the PI3K/Akt signaling and EMT-related factors: β-catenin, Slug, Snail, fibronectin, and vimentin. The expression of gastrokine 1 would increase the expression of E-cadherin [84]. Thus, it is implicated that gastrokine 1 has a major role in GC development by regulating EMT and metastatic cancer cell repression.

26.4.3 EMT, Cancer Stem Cells, and *H. pylori*

Growing evidence that *H. pylori* induced the EMT process has been reported. In particular, it is supposed that CagA of *H. pylori* played an important role in triggering EMT in gastric epithelial cells [50, 53]. CagA can be injected into host gastric epithelial cells via a type IV secretion pilus [85], and this hampers the expressions of junctional adhesion molecules or E-cadherin leading to the destruction of desmosomes or hemidesmosomes. The roles of *H. pylori* in the EMT process have been explained in terms of several transduction pathways [52, 53, 86, 87]. A minority of tumor cells have the potential for self-renewal, differentiation, de-differentiation, and tumor initiation [87]. These cells are called CSCs or cancer stem cell-like cells (CSC-like cells), and they play an essential role in tumor metastasis, invasion, and disease recurrence [87].

26.4.3.1 Promotion of Production of TGF-β- or TNF-α-Inducing Protein

TGF-β is one of the most important triggering factors for EMT, and *H. pylori* infection accelerates the production of TGF-β in human gastric mucosa or gastric epithelial cells [88–90]. *H. pylori* also promotes the production of TNF-α-inducing protein, which produces various cytokines including TNF-α, facilitates the immigration of GC cells, and activates minting leading to the acceleration of EMT [91]. Consequently, chronic infection of *H. pylori* promotes the production of multiple cytokines and prepares the environment to favor EMT.

26.4.3.2 Activation of Snail, Twist, or β-Catenin

CagA binds GSK-3 in a manner similar to Axin and causes it to shift to an insoluble fraction, resulting in reduced GSK-3 activity [92] (Fig. 26.7). It was reported that the production of Snail protein is increased in *H. pylori*-infected epithelium. These results suggest that CagA induces a Snail-mediated EMT via the depletion of GSK-3 [92]. In GC cells cocultured with CagA expression plasmid, CagA activated TWIST1 and vimentin expression and inhibited E-cadherin expression by downregulating programmed cell death factor 4 (PDCD4) [93]. CagA also promoted the mobility of GC cells by regulating PDCD4. In addition, *H. pylori* could activate

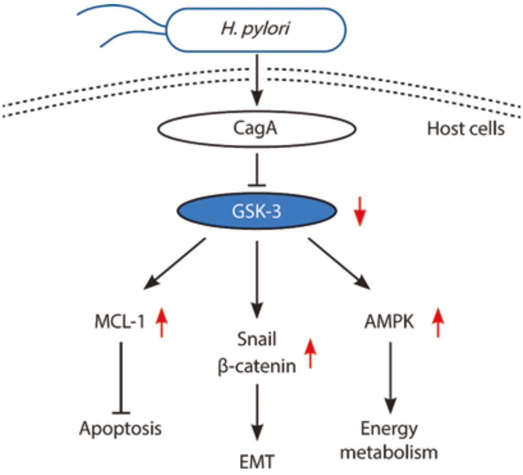

Fig. 26.7 Schematic diagram of CagA-mediated GSK-3 depletion and subsequent activation of EMT and other oncogenic pathways. Following the transduction of CagA into the cells via a type IV secretion system, the cytoplasmic GSK-3 is rapidly insolubilized and downregulated, resulting in the activation of the GSK-3-dependent oncogenic pathway. *GSK3β* glycogen synthase kinase-3β, *MCL-1* myeloid cell leukemia, *EMT* epithelial-mesenchymal transition, *AMPK* AMP-activated protein kinase (adapted from Lee et al. [92])

Wnt/β-catenin signaling pathway via the overexpression of β-catenin, and this finally promotes the EMT event [94, 95]. Authors have recently reported that upregulation of Twist, Snail, Slug, vimentin and downregulation of E-cadherin increased the ability to migrate [96]. In addition, the upregulation of EMT-promoting miRNAs and downregulation of EMT-repressing miRNAs in *H. pylori*-infected mucosa are revealed [78]. How *H. pylori* acts to these miRNAs deserves further study.

26.4.3.3 Hypermethylation of *CDH1* Promoter

Hypermethylation of *CDH1* in breast cancer cells is a part of the EMT process, and this facilitates the metastasis to other organs, endowing more invasive characteristics [97]. *H. pylori* infection promoted the hypermethylation of *CDH1* promoter [98], and more frequent lymph node metastasis or more invasive invasion of tissue was found in the groups with the hypermethylated gene compared to the other group [99]. This suggested that *H. pylori* infection promoted EMT process through hypermethylation of *CDH1* promoter.

26.4.3.4 Association Between Emergence of Cancer Stem Cells and Chronic *H. pylori* Infection

Recent research suggests that cells that undergo EMT obtain the ability to acquire CSC properties [50]. The main source of gastric CSCs includes stem cells and progenitor cells and bone marrow-derived cells [100]. Due to the ability of self-renewal and differentiation into a vast number of cells, CSCs have the property of tumorigenesis induction [101]. Gastric CSCs are primarily generated by CagA-positive *H. pylori* strain infection [102]. High expression of CD44, a compelling marker of CSCs, predisposes cells to the induction of mesenchymal phenotype and EMT [103]. Bessède et al. studied the role of *H. pylori* in the generation of cells with CSC properties, including several gastric epithelial cell lines (AGS, MKN-45, MKN-74) [104]. The role of CagA in the induction of CSC properties was studied on *H. pylori* 7.13 wild-type (WT) *cagA*-positive strain and its knock-out mutants—7.13 *cagA*-negative and 7.13Δ *cagE*-negative *H. pylori* strains. Only the WT (7.13 *cagA*-positive) strain induced mesenchymal changes and EMT in cells; that is, cells infected by *cagA*-positive strains, presented higher expression of mesenchymal markers—CD44, Snail1, Vimentin, or ZEB1, while an expression of epithelial markers—cytokeratin 7 (CK7) or osteopontin (SSP-1) was lowered [104]. The migration and invasion properties of cells were enhanced during infection by *cagA*-positive *H. pylori* strains [50]. The relevance of the infection by *cagA*-positive *H. pylori* strain on EMT in GC was confirmed by higher expression of CD44 and mesenchymal markers in tumor samples. The Wnt/β-catenin signaling pathway was involved in *cagA*-positive *H. pylori* EMT and the induction of CSC properties [105]. Likewise, chronic *cagA*-positive *H. pylori* infection with *N*-nitrosoguanidine (MNNG) stimulation causes CSC features with dysplastic lesions and mesenchymal phenotype [50]. Interestingly, CD44+ cells with CSC features displayed increased CagA half-life [106]. Accumulation of intracellular CagA was confirmed in CD44+ gastric CSCs [107]. *H. pylori*-infected (*cagA*-positive) GC cells exhibit CSC properties via increased expression of surface markers—CD44 and Lgr5 with Nanog, Oct4, and c-myc upregulation [108]. CagA impairs transcription factor CDX1 expression, promoting EMT, and CSC features in gastric epithelial cells [109]. Furthermore, it is reported that tumor necrosis factor-α-inducing protein (Tipα) promotes EMT and CSC-like properties in GC cells through activation of Wnt/β-catenin signaling pathway, thereby accelerating the progression of GC [50].

26.4.4 Clinical Implications of EMT and Cancer Stem Cells

26.4.4.1 Markers for EMT

Markers of established or ongoing EMT are quite consistent. Loss or degradation of proteins associated with epithelial homeostasis, cell polarity, and cell adhesion, such as E-cadherin, RhoA, or

Plakophilin 2, is frequently observed in EMT [110]. The redistribution to the cytoplasm of β-catenin that is involved in cell–cell adhesion is also a hallmark of EMT. The intermediate filament protein vimentin is frequently overexpressed and contributes to cell migration and is thus regarded as a stable marker of EMT. Dysregulated expression of transcription factors, such as Notch1, Slug, Snail, Twist, or Zeb1, has been described in invasive tumors displaying EMT. Table 26.2 represents dysregulated protein markers that have been frequently used in the assessment of EMT [110].

26.4.4.2 Prognostic Factors

Since the metastasis of malignant cancers accounts for the majority of cancer-related deaths, possible correlations between EMT markers and patient prognosis have been intensively studied in various types of tumors. Therefore, most effort has been put into linking the expression of EMT markers to data on patient survival. With respect to GC, significant correlations between the expression of Slug and the lymph node metastasis, lymphovascular invasion, and postoperative TNM stage were revealed [63]. In addition, patients with downregulated E-cadherin or upregulated Slug did not show a favorable prognosis. That is, patients with upregulation of Slug had a lower 5-year survival rate than patients with upregulation of Slug, when their expression of E-cadherin was not changed [111]. The expression of Twist in GC tissue also had positive correlations with the size of tumor, depth of invasion, and lymph node metastasis, and the poor prognosis was more prominent in diffuse-type GC [69]. Positive expression of vimentin is associated with advanced stage and diffuse-type GC. Expression levels of EMT markers Snail and β-catenin were independent predictors for lymph node metastasis and lymphovascular invasion [112]. In addition, overexpression of Snail was found to be associated with lymph node metastasis and poor prognosis in patients with GC [113]. Similarly, loss of E-cadherin expression and upregulation of E-cadherin-suppressing factors are likely to be prognostic factors for GC. However, it may be difficult to yield reliable prognostic information for an individual patient from the expression pattern of EMT markers due to the high variability

Table 26.2 Frequently used protein markers for epithelial-mesenchymal transition (EMT)

Marker	Original function	Tissue
Downregulated in EMT		
α-Catenin	Cell adhesion molecule	Lung
β-Catenin (membrane)	Cell adhesion molecule	Colon, pancreas (NET)
Claudin	Cytoskeletal filament	Esophagus, breast
Cytokeratins	Cell adhesion molecule	Lung, esophagus
E-cadherin	Cell adhesion molecule	Colon, breast, lung, ovary, esophagus, prostate, cervix
Occludin	Cell adhesion molecule	Ovary
Upregulated in EMT		
Brachyury	Transcription factor	Pancreas, breast, lung
β-Catenin (cytoplasm/nucleus)	Transcription factor	Breast, cervix
EGFR	Tyrosine kinase receptor	Cervix
N-cadherin	Cell adhesion molecule	Ovary, prostate
Notch-1	Transcription factor	Prostate
p16^{INK4a}	Cell cycle regulator	Colon, urothelium
Slug	Transcription factor	Breast, ovary
Snail	Transcription factor	Breast, cervix, ovary
TTF-1	Transcription factor	Lung
Twist	Transcription factor	Breast, stomach
Vimentin	Cytoskeletal filament	Breast, esophagus, cervix
ZEB1	Transcription factor	Colon, breast, ovary

Adapted from Steinestel et al. [110]

EMT epithelial-mesenchymal transition, *NET* neuroendocrine tumor, *EGFR* epidermal growth factor receptor, *TTF-1* thyroid transcription factor-1, *ZEB1* zinc finger E-box-binding homeobox 1 of marker expression patterns in different tumor areas in a heterogeneous sample.

26.4.4.3 Cancer Stem Cells

EMT participates in the carcinogenesis process and is involved in the generation of CSC. In one study [104], gastric epithelial cells that were cocultured with a *cagA*-positive *H. pylori* strain were transfected with CagA expression vectors. The expression of epithelial and mesenchymal markers showed that *H. pylori*, via CagA, is responsible for an EMT phenotype associated with an increase in mesenchymal markers and CD44 expression, a known gastric CSC marker [104]. Moreover, infection led to an increased ability to migrate, to invade, and to form tumor spheres. Cell-sorting experiments showed that only the CD44^{high} cells induced by *H. pylori* infection displayed the mesenchymal phenotype and CSC properties in vitro and had higher tumorigenic properties than CD44^{low} cells in xenografted mice. This result suggests that a certain association between EMT and cancer and the infection with *cagA*-positive *H. pylori* induces EMT-like changes and the emergence of CD44^{high} cells with CSC properties.

26.4.4.4 Targets for the Cancer Treatments

Since stem cell-like tumor cells bear considerable resistance to conventional therapies and the markers for EMT have been identified in a significant proportion of these cells as described above, much effort has been made to develop antineoplastic therapies that directly target EMT.

Most of the therapeutic approaches in this field are aimed at blocking EMT master genes directly or inhibiting kinase signaling pathways to switch off the EMT master gene expression. They have been developed in hepatic cell cancer, colorectal cancer, and pancreatic cancer with respect to the gastroenterological part.

Resveratrol, a dietary polyphenol, downregulated expression of EMT master genes (*Zeb1*, *Snail*, and *Slug*) and impaired cancer stem cell self-renewal capacity, tumor growth, and invasion in a mouse model of pancreatic ductal adenocarcinoma [114]. In recent years, the role of small, noncoding RNAs in the EMT process has also been further elucidated. Methylation-dependent expression changes in levels of miR-200c regulate invasion and metastasis in cancer via altered miR-200c target gene expression; miR-200 family also activates cyclin D1 leading to loss of mesenchymal characteristics of EMT-progressed epithelium [115–119]. MiR-let-7f is known to be a tumor-suppressing factor targeting MYH9, a metastasis-related gene [120]. MiR-7 is also downregulated in GC tissue like miR-let-7f, and the expression of miR-7 showed an inverse correlation with metastatic spread or invasion of GC cells [121]. Taken together, antineoplastic agents using miRNAs could suppress metastasis of gastric tumors.

26.5 Conclusions

The EMT is characterized by the morphologic and molecular changes: loss of E-cadherin, change to spindle shape, and gain of the ability to migrate. It is critical for embryonic development, wound healing, and fibrotic disease. Moreover, recent studies have reported that aberrant EMT activation in the human organ is closely associated with carcinogenesis and tumor progression. There are a number of proteins that indicate the stage of EMT (i.e., E-cadherin, N-cadherin, and vimentin). The transcriptional proteins such as E-cadherin transcriptional repressors could be also markers for EMT. The EMT process is under tight control of multiple signal pathways including TGF-β-related Smad pathway, one of the important axes. Therefore, downregulation of E-cadherin function due to mutation, deletion, CpG hypermethylation, and SNAIL-mediated transcriptional repression of the *CDH1* gene leads to EMT. Dysregulation of proteins in EMT pathways and epigenetic mechanisms could be involved in the progression of EMT.

Growing evidence has been recently reported that EMT endows cells with not only migratory and invasive characteristics but also stem cell properties. Increased expression of mesenchymal

EMT markers was closely connected with the emergence of cancer stem cells. Especially, it has been reported that *H. pylori* infection, one of the major gastric carcinogens, could increase mesenchymal EMT markers and promote migration activity. This implicated that EMT induced by *H. pylori* may be a cause of gastric carcinogenesis by triggering gastric CSC. Recently, it has been reported that *cagA*-positive *H. pylori* infection induces EMT-like changes and the emergence of CD44^{high} cells with CSC properties. Furthermore, it was suggested that Tipα promotes EMT and CSC-like properties in GC cells through activation of Wnt/β-catenin signaling pathway, thereby accelerating the progression of GC [50]. Taken together, understanding the role of EMT and CSC could be helpful for the early detection and effective treatment of GC.

References

1. Wu J, Mlodzik M. A quest for the mechanism regulating global planar cell polarity of tissues. Trends Cell Biol. 2009;19:295–305.
2. Beyer Nardi N, da Silva ML. Mesenchymal stem cells: isolation, in vitro expansion and characterization. Handb Exp Pharmacol. 2006;174:249–82.
3. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol. 2006;7:131–42.
4. Kong D, Li Y, Wang Z, Sarkar FH. Cancer stem cells and epithelial-to-mesenchymal transition (EMT)-phenotypic cells: are they cousins or twins? Cancers. 2011;3:716–29.
5. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol. 2014;15:178–96.
6. Kalluri R. EMT: when epithelial cells decide to become mesenchymal-like cells. J Clin Invest. 2009;119:1417–9.
7. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009;139:871–90.
8. Iwatsuki M, Mimori K, Yokobori T, Ishi H, Beppu T, Nakamori S. Epithelial-mesenchymal transition in cancer development and its clinical significance. Cancer Sci. 2010;101:293–9.
9. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119:1420–8.
10. Savagner P, Boyer B, Valles AM, Jouanneau J, Thiery JP. Modulations of the epithelial phenotype during embryogenesis and cancer progression. Cancer Treat Res. 1994;71:229–49.
11. Moustakas A, Heldin CH. Signaling networks guiding epithelial-mesenchymal transitions during embryogenesis and cancer progression. Cancer Sci. 2007;98:1512–20.
12. Wells RG. Epithelial to mesenchymal transition in liver fibrosis: here today, gone tomorrow? Hepatology. 2010;51:737–40.
13. López-Novoa JM, Nieto MA. Inflammation and EMT: an alliance towards organ fibrosis and cancer progression. EMBO Mol Med. 2009;1:303–14.
14. Park SM, Kim SM, Han JH. The role of epithelial-mesenchymal transition in the gastroenterology. Korean J Gastroenterol. 2010;56:69–77.
15. Fidler IJ, Poste G. The "seed and soil" hypothesis revisited. Lancet Oncol. 2008;9:808–12.
16. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer. 2002;2:442–54.
17. Thompson EW, Haviv I. The social aspects of EMT-MET plasticity. Nat Med. 2011;17:1048–9.
18. Bissell MJ, Radisky DC, Rizki A, Weaver VM, Petersen OW. The organizing principle: microenvironmental influences in the normal and malignant breast. Differentiation. 2002;70:537–46.
19. Jechlinger M, Grunert S, Beug H. Mechanisms in epithelial plasticity and metastasis: insights from 3D cultures and expression profiling. J Mammary Gland Biol Neoplasia. 2002;7:415–32.
20. Smit MA, Peeper DS. Deregulating EMT and senescence: double impact by a single twist. Cancer Cell. 2008;14:5–7.
21. Weinberg RA. Twisted epithelial-mesenchymal transition blocks senescence. Nat Cell Biol. 2008;10:1021–3.
22. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell. 2003;113:685–700.
23. Niessen K, Fu YX, Chang L, Hoodless PA, McFadden D, Karsan A. Slug is a direct notch target required for initiation of cardiac cushion cellularization. J Cell Biol. 2008;182:315–25.
24. Medici D, Hay ED, Olsen BR. Snail and slug promote epithelial-mesenchymal transition through beta-catenin-T-cell factor-4-dependent expression of transforming growth factor-beta3. Mol Biol Cell. 2008;19:4875–87.
25. Kokudo T, Suzuki Y, Yoshimatsu Y, Yamazaki T, Watabe T, Miyazono K. Snail is required for TGFβ-induced endothelial-mesenchymal transition of embryonic stem cell-derived endothelial cells. J Cell Sci. 2008;121:3317–24.
26. Willis BC, Borok Z. TGF-β-induced EMT: mechanisms and implications for fibrotic lung disease. Am J Physiol Cell Mol Physiol. 2007;293:L525–34.
27. Gupta PB, Mani S, Yang J, Hartwell K, Weinberg RA. The evolving portrait of cancer metastasis. Cold Spring Harb Symp Quant Biol. 2005;70:291–7.

28. Yang J, Mani SA, Weinberg RA. Exploring a new twist on tumor metastasis. Cancer Res. 2006;66:4549–52.
29. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. 2008;133:704–15.
30. Hartwell KA, Muir B, Reinhardt F, Carpenter AE, Sgroi DC, Weinberg RA. The Spemann organizer gene, Goosecoid, promotes tumor metastasis. Proc Natl Acad Sci U S A. 2006;103:18969–74.
31. Bierie B, Moses HL. Tumour microenvironment: TGFβ: the molecular Jekyll and Hyde of cancer. Nat Rev Cancer. 2006;6:506–20.
32. Oft M, Heider KH, Beug H. TGF-beta signaling is necessary for carcinoma cell invasiveness and metastasis. Curr Biol. 1998;8:1243–52.
33. Song J. EMT or apoptosis: a decision for TGF-beta. Cell Res. 2007;17:289–90.
34. Miyazono K, ten Dijke P, Heldin CH. TGF-beta signaling by Smad proteins. Adv Immunol. 2000;75:115–57.
35. Heldin CH, Miyazono K, ten Dijke P. TGF-beta signaling from cell membrane to nucleus through SMAD proteins. Nature. 1997;390:465–71.
36. Roberts AB, Tian F, Byfield SDC, Stuelten C, Ooshima A, Saika S, et al. Smad3 is key to TGF-beta-mediated epithelial-to-mesenchymal transition, fibrosis, tumor suppression and metastasis. Cytokine Growth Factor Rev. 2006;17:19–27.
37. Piek E, Moustakas A, Kurisaki A, Heldin CH, Dijke PT. TGF-(beta) type I receptor/ALK-5 and Smad proteins mediate epithelial to mesenchymal transdifferentiation in NMuMG breast epithelial cells. J Cell Sci. 1999;112:4557–68.
38. Bhowmick NA, Ghiassi M, Bakin A, Aakre M, Lundquist CA, Engel ME, et al. Transforming growth factor-beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism. Mol Biol Cell. 2001;12:27–36.
39. Lee YH, Albig AR, Regner MA, Schiemann BJ, Schiemann WP. Fibulin-5 initiates epithelial-mesenchymal transition (EMT) and enhances EMT induced by TGF-beta in mammary epithelial cells via a MMP-dependent mechanism. Carcinogenesis. 2008;29:2243–51.
40. Lehmann K, Janda E, Pierreux CE, Rytömaa M, Schulze A, McMahon M, et al. Raf induces TGFbeta production while blocking its apoptotic but not invasive responses: a mechanism leading to increased malignancy in epithelial cells. Genes Dev. 2000;14:2610–22.
41. Gotzmann J, Huber H, Thallinger C, Wolschek M, Jansen B, Schulte-Hermann R, et al. Hepatocytes convert to a fibroblastoid phenotype through the cooperation of TGF-beta1 and ha-Ras: steps towards invasiveness. J Cell Sci. 2002;115:1189–202.
42. Oft M, Peli F, Rudaz C, Schwarz H, Beug H, Reichmann E. TGF-beta1 and ha-Ras collaborate in modulating the phenotypic plasticity and invasiveness of epithelial tumor cells. Genes Dev. 1996;10:2462–77.
43. Janda E, Lehmann K, Killisch I, Jechlinger M, Herzig M, Downward J, et al. Ras and TGF[beta] cooperatively regulate epithelial cell plasticity and metastasis: dissection of Ras signaling pathways. J Cell Biol. 2002;156:299–313.
44. Cui W, Fowlis DJ, Bryson S, Duffie E, Ireland H, Balmain A, et al. TGFbeta1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice. Cell. 1996;86:531–42.
45. Neil JR, Johnson KM, Nemenoff RA, Schiemann WP. Cox-2 inactivates Smad signaling and enhances EMT stimulated by TGF-beta through a PGE2-dependent mechanisms. Carcinogenesis. 2008;29:2227–35.
46. Stockinger A, Eger A, Wolf J, Beug H, Foisner R. E-cadherin regulates cell growth by modulating proliferation-dependent beta-catenin transcriptional activity. J Cell Biol. 2001;154:1185–96.
47. Gottardi CJ, Wong E, Gumbiner BM. E-cadherin suppresses cellular transformation by inhibiting beta-catenin signaling in an adhesion-independent manner. J Cell Biol. 2001;153:1049–60.
48. Kim K, Lu Z, Hay ED. Direct evidence for a role of beta-catenin/LEF-1 signaling pathway in induction of EMT. Cell Biol Int. 2002;26:463–76.
49. Timmerman LA, Grego-Bessa J, Raya A, Bertrán E, Pérez-Pomares JM, Díez J, et al. Notch promotes epithelial mesenchymal transition during cardiac development and oncogenic transformation. Genes Dev. 2004;18:99–115.
50. Baj J, Korona-Głowniak I, Forma A, Maani A, Sitarz E, Rahnama-Hezavah M, et al. Mechanisms of the epithelial-mesenchymal transition and tumor microenvironment in *Helicobacter pylori*-induced gastric cancer. Cell. 2020;9:1055.
51. Katoh M. Epithelial-mesenchymal transition in gastric cancer (review). Int J Oncol. 2005;27:1677–83.
52. Chen G, Tang N, Wang C, Xiao L, Yu M, Zhao L, et al. TNF-α-inducing protein of *Helicobacter pylori* induces epithelial-mesenchymal transition (EMT) in gastric cancer cells through activation of IL-6/STAT3 signaling pathway. Biochem Biophys Res Commun. 2017;484:311–7.
53. Sougleri IS, Papadakos KS, Zadik MP, Vavagianni MM, Andreas F, Mentis AF, et al. *Helicobacter pylori* CagA protein induces factors involved in the epithelial to mesenchymal transition (EMT) in infected gastric epithelial cells in an EPIYA-phosphorylation-dependent manner. FEBS. 2016;283:206–20.
54. Magalhães A, Marcos NT, Carvalho AS, David L, Figueiredo C, Bastos J, et al. *Helicobacter pylori* cag pathogenicity island-positive strains induce syndecan-4 expression in gastric epithelial cells. FEMS Immunol Med Microbiol. 2009;56:223–32.
55. Backert S, Neddermann M, Maubach G, Naumann M. Pathogenesis of *Helicobacter pylori* infection. Helicobacter. 2016;21(Suppl 1):19–25.

56. Hu B, Khara P, Song L, Lin AS, Frick-Cheng AE, Harvey ML, et al. In situ molecular architecture of the *Helicobacter pylori* cag type IV secretion system. MBio. 2019;10:e00849–19.
57. Lee MH, Yang JY, Cho Y, Woo HJ, Kwon HJ, Kim DH, et al. Inhibitory effects of menadione on *Helicobacter pylori* growth and *Helicobacter pylori*-induced inflammation via NF-κB inhibition. Int J Mol Sci. 2019;20:1169.
58. Roskoski R. ERK1/2 MAP kinases: structure, function, and regulation. Pharm Res. 2012;66:105–43.
59. Buti L, Ruiz-Puig C, Sangberg D, Lu X. CagA–ASPP2 complex mediates loss of cell polarity and favors *H. pylori* colonization of human gastric organoids. Proc Natl Acad Sci U S A. 2020;117:2645–55.
60. Bacon S, Seeneevassen L, Fratacci A, Rose F, Tiffon C, Sifré F, et al. Nrf2 downregulation contributes to epithelial-to-mesenchymal transition in *Helicobacter pylori*-infected cells. Cancers (Basel). 2022;14:4316.
61. Hayes JD, Dinkova-Kostova AT. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem Sci. 2014;39:199–218.
62. Graziano F, Humar B, Guilford P. The role of the E-cadherin gene (CDH1) in diffuse gastric cancer susceptibility: from the laboratory to clinical practice. Ann Oncol. 2003;14:1705–13.
63. Castro Alves C, Rosivatz E, Schott C, Hollweck R, Becker I, Sarbia M, Carneiro F, et al. Slug is overexpressed in gastric carcinomas and may act synergistically with SIP1 and snail in the down-regulation of E-cadherin. J Pathol. 2007;211:507–15.
64. He H, Chen W, Wang X, Wang C, Liu F, Shen Z, et al. Snail is an independent prognostic predictor for progression and patient survival of gastric cancer. Cancer Sci. 2012;103:1296–303.
65. Rosivatz E, Becker KF, Kremmer E, Schott C, Blechschmidt K, Höfler H, et al. Expression and nuclear localization of snail, an E-cadherin repressor, in adenocarcinomas of the upper gastrointestinal tract. Virchows Arch. 2006;448:277–87.
66. Ngo HK, Lee HG, Piao JY, Zhong X, Lee HN, Han HJ, et al. *Helicobacter pylori* induces snail expression through ROS-mediated activation of Erk and inactivation of GSK-3β in human gastric cancer cells. Mol Carcinog. 2016;55:2236–46.
67. Lee SD, Jeong H, Hwang BR, Yu BM, Cho Y, Nam KT, et al. *Helicobacter pylori* promotes epithelial-to-mesenchymal transition by downregulating CK2β in gastric cancer cells. Biochim Biophys Acta Mol Basis Dis. 2022;1869:16658.
68. Yang Z, Zhang X, Gang H, Li X, Li Z, Wang T, et al. Up-regulation of gastric cancer cell invasion by twist is accompanied by N-cadherin and fibronectin expression. Biochem Biophys Res Commun. 2007;358:925–30.
69. Yan-Qi Z, Xue-Yan G, Shuang H, Yu C, Fu-Lin G, Fei-Hu B, et al. Expression and significance of TWIST basic helix–loop–helix protein over-expression in gastric cancer. Pathology. 2007;39:470–5.
70. Sung CO, Lee KW, Han S, Kim SH. Twist1 is up-regulated in gastric cancer-associated fibroblasts with poor clinical outcomes. Am J Pathol. 2011;179:1827–38.
71. Zhang J, Ma L. MicroRNA control of epithelial-mesenchymal transition and metastasis. Cancer Metastasis Rev. 2012;31:653–62.
72. Sun Q, Gu H, Zeng Y, Xia Y, Wang Y, Jing Y, et al. Hsa-mir-27a genetic variant contributes to gastric cancer susceptibility through affecting miR-27a and target gene expression. Cancer Sci. 2010;101:2241–7.
73. Liu T, Tang H, Lang Y, Liu M, Li X. MicroRNA-27a functions as an oncogene in gastric adenocarcinoma by targeting prohibitin. Cancer Lett. 2008;273:233–42.
74. Bracken CP, Gregory PA, Kolesnikoff N, Bert AG, Wang J, Shannon MF, et al. A double- negative feedback loop between ZEB1–SIP1 and the microRNA-200 family regulates epithelial-mesenchymal transition. Cancer Res. 2008;68:7846–54.
75. Ma L, Young J, Prabhala H, Pan E, Mestdagh P, Muth D, et al. miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nat Cell Biol. 2010;12:247–56.
76. Kong W, Yang H, He L, Zhao JJ, Coppola D, Dalton WS, et al. MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributions to epithelial cell plasticity by targeting RhoA. Mol Cell Biol. 2008;28:6773–84.
77. Wang FE, Zhang C, Maminishkis A, Dong L, Zhi C, Li R, et al. MicroRNA-204/211 alters epithelial physiology. FASEB J. 2010;24:1552–71.
78. Noto JM, Peek RM. The role of microRNAs in *Helicobacter pylori* pathogenesis and gastric carcinogenesis. Front Cell Infect Microbiol. 2012;1:21.
79. Park Y, Kim JW, Kim DS, Kim EB, Park SJ, Park JY, et al. The bone morphogenesis protein-2 (BMP-2) is associated with progression to metastatic disease in gastric cancer. Cancer Res Treat. 2008;40:127–32.
80. Kang MH, Kim JS, Seo JE, Oh SC, Yoo YA. BMP2 accelerates the motility and invasiveness of gastric cancer cells via activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. Exp Cell Res. 2010;316:24–37.
81. Kang MH, Oh SC, Lee HJ, Kang HN, Kim JL, Kim JS, et al. Metastatic function of BMP-2 in gastric cancer cells: the role of PI3K/AKT, MAPK, the NF-κB pathway, and MMP-9 expression. Exp Cell Res. 2011;317:1746–62.
82. Jung H, Jun KH, Jung JH, Chin HM, Park WB. The expression of claudin-1, claudin-2, claudin-3, and claudin-4 in gastric cancer tissue. J Surg Res. 2011;167:e185–91.
83. Agarwal R, Mori Y, Cheng Y, Jin Z, Olaru AV, Hamilton JP, et al. Silencing of claudin-11 is asso-

ciated with increased invasiveness of gastric cancer cells. PLoS One. 2009;4:e8002.

84. Yoon JH, Kang YH, Choi YJ, Park IS, Nam SW, Lee JY, et al. Gastrokine 1 functions as a tumor suppressor by inhibition of epithelial-mesenchymal transition in gastric cancers. J Cancer Res Clin Oncol. 2011;137:1697–704.
85. Kwok T, Zabler D, Urman S, Rohde M, Hartig R, Wessler S, et al. *Helicobacter* exploits integrin for type IV secretion and kinase activation. Nature. 2007;449:862–6.
86. Zhang Q, Ding J, Liu J, Wang W, Zhang F, Wang J, et al. *Helicobacter pylori*-infected MSCs acquire a pro-inflammatory phenotype and induce human gastric cancer migration by promoting EMT in gastric cancer cells. Oncol Lett. 2016;11:449–57.
87. Guo K, Duan J, Lu J, Xiao L, Han L, Zeng S, et al. Tumor necrosis factor-α-inducing protein of *Helicobacter pylori* promotes epithelial-mesenchymal transition and cancer stem-like cells properties via activation of Wnt/β-catenin signaling pathway in gastric cancer cells. Pathog Dis. 2022;80:ftac025.
88. Beswick EJ, Pinchuk IV, Earley RB, Schmitt DA, Reyes VE. Role of gastric epithelial cell-derived transforming growth factor β in reduced CD4+ T cell proliferation and development of regulatory T cells during *Helicobacter pylori* infection. Infect Immun. 2011;79:2737–45.
89. Smythies LE, Sellers M, Clements RH, Mosteller-Barnum M, Meng G, Benjamin WH, et al. Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. J Clin Invest. 2005;115:66–75.
90. Lindholm C, Quiding-Järbrink M, Lönroth H, Hamlet A, Svennerholm AM. Local cytokine response in *Helicobacter pylori*-infected subjects. Infect Immun. 1998;66:5964–71.
91. Watanabe T, Takahashi A, Suzuki K, Kurusu-Kanno M, Yamaguchi K, Fujiki H, et al. Epithelial-mesenchymal transition in human gastric cancer cell lines induced by TNF-α-inducing protein of *Helicobacter pylori*. Int J Cancer. 2014;10:2373–82.
92. Lee DG, Kim HS, Lee YS, Kim S, Cha SY, Ota I, et al. *Helicobacter pylori* CagA promotes snail-mediated epithelial–mesenchymal transition by reducing GSK-3 activity. Nat Commun. 2014;5:4423.
93. Yu H, Zeng J, Liang X, Wang W, Zhou Y, Sun Y, et al. *Helicobacter pylori* promotes epithelial–mesenchymal transition in gastric cancer by downregulating programmed cell death protein 4. PLoS One. 2014;9:e105306.
94. Murata-Kamiya N, Kurashima Y, Teishikata Y, Yamahashi Y, Saito Y, Higashi H, et al. *Helicobacter pylori* CagA interacts with E-cadherin and deregulates the beta-catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells. Oncogene. 2007;26:4617–26.
95. Franco AT, Israel DA, Washington MK, Krishna U, Fox JG, Rogers AB, et al. Activation of beta-catenin by carcinogenic *Helicobacter pylori*. Proc Natl Acad Sci U S A. 2005;102:10646–51.
96. Chang H, Kim N, Park JH, Nam RH, Choi YJ, Park SM, et al. *Helicobacter pylori* might induce TGF-β1-mediated EMT by means of cagE. Helicobacter. 2015;20:438–48.
97. Lombaerts M, van Wezel T, Philippo K, Dierssen JW, Zimmerman RM, Oosting J, et al. E-cadherin transcriptional downregulation by promoter methylation but not mutation is related to epithelial-to-mesenchymal transition in breast cancer cell lines. Br J Cancer. 2006;94:661–71.
98. Perri F, Cotugno R, Piepoli A, Merla A, Quitadamo M, Gentile A, et al. Aberrant DNA methylation in non-neoplastic gastric mucosa of *H. pylori* infected patients and effect of eradication. Am J Gastroenterol. 2007;102:1361–71.
99. Chan AO, Lam SK, Wong BC, Wong WM, Yuen MF, Yeung YH, et al. Promoter methylation of E-cadherin gene in gastric mucosa associated with *Helicobacter pylori* infection and in gastric cancer. Gut. 2003;52:502–6.
100. Mentis AA, Boziki M, Grigoriadis N, Papavassiliou AG. *Helicobacter pylori* infection and gastric cancer biology: tempering a double-edged sword. Cell Mol Life Sci. 2019;76:2477–86.
101. Toh TB, Lim JJ, Chow EKH. Epigenetics in cancer stem cells. Mol Cancer. 2017;16:1–20.
102. Wroblewski LE, Choi E, Petersen C, Delgado AG, Piazuelo MB, Romero-Gallo J, et al. Targeted mobilization of Lrig1+ gastric epithelial stem cell populations by a carcinogenic helicobacter pylori type IV secretion system. Proc Natl Acad Sci U S A. 2019;116:19652–8.
103. Morath I, Hartmann T, Orian-Rousseau V. CD44: more than a mere stem cell marker. Int J Biochem Cell Biol. 2016;81:166–73.
104. Bessède E, Staedel C, Acuña Amador LA, Nguyen PH, Chambonnier L, Hatakeyama M, et al. *Helicobacter pylori* generates cells with cancer stem cell properties via epithelial–mesenchymal transition-like changes. Oncogene. 2014;33:4123–31.
105. Tsugawa H, Suzuki H, Saya H, Hatakeyama M, Hirayama T, Hirata K, et al. Reactive oxygen species-induced autophagic degradation of *Helicobacter pylori* CagA is specifically suppressed in cancer stem-like cells. Cell Host Microbe. 2012;12:764–77.
106. Amieva M, Peek RM. Pathobiology of *Helicobacter pylori*—induced gastric cancer. Gastroenterology. 2016;150:64–78.
107. Yong X, Tang B, Xiao YF, Xie R, Qin Y, Luo G, et al. *Helicobacter pylori* upregulates Nanog and Oct4 via Wnt/β-catenin signaling pathway to promote cancer stem cell-like properties in human gastric cancer. Cancer Lett. 2016;374:292–303.
108. Choi SI, Yoon C, Park MR, Lee D, Kook MC, Lin JX, et al. CDX1 expression induced by CagA-expressing *Helicobacter pylori p*romotes gastric tumorigenesis. Mol Cancer Res. 2019;17:2169–83.

109. Yu FX, Zhao B, Guan KL. Hippo pathway in organ size control, tissue homeostasis, and cancer. Cell. 2015;163:811–28.
110. Steinestel K, Eder S, Schrader AJ, Steinestel J. Clinical significance of epithelial-mesenchymal transition. Clin Transl Med. 2014;3:17–21.
111. Uchikado Y, Okumura H, Ishigami S, Setoyama T, Matsumoto M, Owaki T, et al. Increased slug and decreased E-cadherin expression is related to poor prognosis in patients with gastric cancer. Gastric Cancer. 2011;14:41–9.
112. Fuyuhiro Y, Yashiro M, Noda S, Kashiwagi S, Matsuoka J, Doi Y, et al. Clinical significance of vimentin-positive gastric cancer cells. Anticancer Res. 2010;30:5239–43.
113. Shin NR, Jeong EH, Choi CI, Moon HJ, Kwon CH, Chu IS, et al. Overexpression of snail is associated with lymph node metastasis and poor prognosis in patients with gastric cancer. BMC Cancer. 2012;12:521.
114. Kim MA, Lee HS, Lee HE, Kim JH, Yang HK, Kim WH. Prognostic importance of epithelial–mesenchymal transition-related protein expression in gastric carcinoma. Histopathology. 2009;54:442–51.
115. Park SM, Gaur AB, Lengyel E, Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev. 2008;22:894–907.
116. Burk U, Schubert J, Wellner U, Schmalhofer O, Vincan E, Spaderna S, et al. A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. EMBO Rep. 2008;9:582–9.
117. Chang YJ, Wu MS, Lin JT, Pestell RG, Blaser MJ, Chen CC. Mechanisms for *Helicobacter pylori* CagA-induced cyclin D1 expression that affect cell cycle. Cell Microbiol. 2006;8:1740–52.
118. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol. 2008;10:593–601.
119. Christoffersen NR, Silahtaroglu A, Orom UA, Kauppinen S, Lund AH. miR-200b mediates post-transcriptional repression of ZFHX1B. RNA. 2007;13:1172–8.
120. Liang S, He L, Zhao X, Miao Y, Gu Y, Guo C, et al. MicroRNA let-7f inhibits tumor invasion and metastasis by targeting MYH9 in human gastric cancer. PLoS One. 2011;6:e18409.
121. Zhao X, Dou W, He L, Liang S, Tie J, Liu C, et al. MicroRNA-7 functions as an anti-metastatic microRNA in gastric cancer by targeting insulin-like growth factor-1 receptor. Oncogene. 2013;32:1363–72.

Gastric Cancer: ABO Blood Type

27

Nayoung Kim and Sooyeon Oh

Abstract

Human ABO blood type antigens exhibit alternative phenotypes and genetically derived glycoconjugate structures that are located on the red cell surface play an active role in the cells' physiology and pathology. ABO blood type has been known as a genetic factor which affects the development of gastric cancer. However, the mechanism is still uncertain. There is experimental evidence which suggests the role of ABO blood type in the infection of *Helicobacter pylori* (*H. pylori*). Molecular mimicry was shown with Lewis antigens, with which *H. pylori* can evade the host immune system. Blood group antigens were observed to act as an adhesion molecule onto which *H. pylori* attach. Nonsecretors compared with secretors lack "decoys"; therefore, nonsecretors are more vulnerable to intruders. These findings can be clues for future studies.

Keywords

Helicobacter pylori · Blood group antigen
Gastric cancer

27.1 Introduction

Associations between the blood type and disease have been studied since the early 1900s when researchers determined the inheritance of antibodies and antigens. However, due to lack of antigens in some blood groups, there have been some contentious issues with the association between the ABO blood group and vulnerability to certain infectious and noninfectious diseases [1]. For instance, histo-blood group carbohydrates are proposed to influence the risk of acquiring this pathogen via effects on adhesion to the gastric mucosa [1]. Recently, there was a nice meta-analysis regarding the relationship between ABO blood groups and risk of *Helicobacter pylori* (*H. pylori*) infection [2]. So far, blood type has been associated with the risk of gastric cancer (GC) [3–6]. In addition, recent publications suggested the possible association between esophageal squamous cell carcinoma [7], breast cancer [8], and pancreatic cancer [9–12] and ABO blood groups, suggesting the impact of ABO blood groups on the tumor microenvironment. Various factors, both environmental and genetic factors, contribute to the development of GC. ABO blood type is one of such genetic factors. In a textbook

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of internal medicine by Cecil, blood group A was classified as a risk factor of GC [13]. In the GC risk index provided by the Harvard School of Public Health, blood group A was included as a risk factor. However, the mechanism how blood group A increases the risk of GC is uncertain but seems related to other genetic factors [14]. The study which provided the foundation of the risk index program proposed the relative risk of blood group A as 1.2 [15]. In this chapter the role of ABO blood type in the infection of *H. pylori* and development of GC is briefly reviewed.

27.2 The Association of ABO Blood Group with Different Diseases

Oligosaccharide structures specific to the antigens define the blood type. These carbohydrate components are perceived as extraneous by the immune system which produce antibodies to them [16]. The presence and absence of antigens in some blood types result in blood membrane alterations in both morphology and function [1]. The functions dependent on the structure of blood types can associate the blood groups with diseases and health [17, 18]. In addition to red blood cells (RBCs), blood group antigens can be found on leukocytes, certain tissues, plasma proteins, platelets, and various cell surface enzymes [19]. Furthermore, blood group antigens can also be present in body fluids such as sweat, saliva, breast milk, seminal fluid, urine, gastric secretions, and amniotic fluid with soluble form [20, 21]. The glycoconjugate structures on RBCs have many functions including receptors for exogenous ligands, viruses, bacteria, and parasites, transporters, channels, structural proteins, adhesion molecules, and enzymes [22]. The exact mechanisms that would explain the associations between blood group antigens and disease in adhesion molecules have not yet been identified [1]. However, an unexpected number of these structures contribute to normal RBC development; some act as cell adhesion molecules (CAMs), and some play a role in human diseases [23, 24]. The susceptibility to various diseases, such as cancer, cardiovascular diseases, infections, and hematologic disorders, has been linked with ABO blood groups [25]. Mostly A and B antigens are secreted by the cells and are found in human blood circulation. Therefore, nonsecretors are at risk to a variety of infections [26]. The likely pathogenesis for this vulnerability is that many organisms may bind to polysaccharides on the cell surface but soluble blood group antigens may block this binding [27, 28]. On the one hand, there was a report [29] that the ABO alternative phenotype can prevent the species carrying it from being threatened by a pathogen using a given carbohydrate as a receptor. On the other hand, the ABO polymorphism leads to a polymorphic production of anti-A and anti-B natural antibodies, which potentially protect individuals from several infectious agents expressing A and B motifs [30]. Moreover, blood group AB individuals were found to be susceptible to an increased risk of cognitive impairment, which was independent of geographic region, age, race, and sex [1]. Early etiological studies indicated that blood type O has a connection with increased incidence of cholera, plague, tuberculosis infections, and mumps, whereas blood type A is linked with increased incidence of smallpox and *Pseudomonas aeruginosa* infection; blood type B is also associated with increased incidence of gonorrhea, tuberculosis, and *Streptococcus pneumoniae*, *E. coli*, and salmonella infections; and blood type AB is associated with increased incidence of smallpox and *E. coli* and salmonella infections [1]. Higher incidence of cancers in the stomach, ovaries, salivary glands, cervix, uterus, and colon/rectum was common in blood type A people than in blood type O people [1]. The link between the ABO blood type and thromboembolic diseases and bleeding risk is intervened by the glycosyltransferase activity and plasma levels and biologic activity of vWF (Von Willebrand factor), a carrier protein for coagulation factor VIII, which is low in O type [1]. Taken together, ABO phenotype suggested that genetically determined human ABO blood groups were correspondingly linked with an increased risk of various infectious and noninfectious diseases including *H. pylori*. So far, investigations on the molecular level of ABO blood groups and their association with *H. pylori*-associated diseases are slowly undergoing.

27.3 Association Between ABO Blood Groups and *H. pylori* Infection

H. pylori is a Gram-negative spiral-shaped pathogenic bacterium which inhabits the human gastric mucosa. The bacterium is present in approximately half of the world's population [31, 32], but it causes symptomatic disease (peptic ulcer disease and gastric malignancy) in only 20% of those infected. Understanding who is likely to become infected and develop disease is extremely important, especially with increasingly common antibiotic-resistant strains and the lack of an effective vaccine [33]. One genetically determined trait with known polymorphic expression between individuals and populations that has attracted interest as a potential risk factor for *H. pylori* infection is ABO blood group [34]. This premise was developed from previous studies showing a higher frequency of blood group O among patients with duodenal ulcer [35]. Since duodenal ulcer disease is associated with antral *H. pylori* infection in 90–100% of cases, blood group O might also be a risk factor for acquiring *H. pylori* infection [2]. ABO blood groups have also been investigated as risk factors for *H. pylori*-associated GC; however, there are conflicting studies due to multiple confounding effects [36]. Blood group antigens are receptors for toxins, parasites, and bacteria, where they can facilitate colonization or invasion or evade host clearance mechanisms [37]. Previous studies demonstrated that blood group antigen-binding adhesion (BabA) mediates adherence of *H. pylori* to human Lewis b (α-1,3/4-difucosylated) blood group antigens on gastric epithelial cells [38, 39]. During the last few decades, evidence for the role of ABO groups as potential risk factors for *H. pylori* infection has emerged from animal model studies and epidemiological data [40–42]. *H. pylori* infection was found in some studies to be positively associated with O blood group [40, 43], while others found no correlations [41, 42, 44, 45]. Recently, there was a nice systematic review and meta-analysis of the relevant epidemiologic literature which aimed to quantify the association between ABO blood group and *H. pylori* infection status [2]. This study showed that individuals with O blood group were more likely to be infected with *H. pylori* (pooled OR 1.163; 95% CI, 1.074–1.259; $p < 0.001$) [2] (Fig. 27.1), while individuals with B blood group (Fig. 27.2) and AB blood group (Fig. 27.3) were less likely to be infected with *H. pylori* (OR 0.831; 95% CI, 0.738–0.935; $p = 0.002$, and OR 0.709; 95% CI, 0.605–0.832; $p < 0.001$, respectively) [2]. The results from this meta-analysis of observational studies suggest an estimated 16.3% increased odds of *H. pylori* infection among individuals with the O blood group [2]. If this observed association is causal, a better understanding of the underlying mechanisms could provide indications to potential prevention strategies for *H. pylori* infection [2].

Model	Study name	Statistics for each study				
		Odds ratio	Lower limit	Upper limit	Z-Value	*p*-Value
	Niv, 1996	0.636	0.345	1.172	-1.451	0.147
	Valliani, 2013	3.143	1.320	7.482	2.588	0.010
	Umlauft, 1996	1.032	0.675	1.577	0.144	0.885
	Rasmi, 2011	1.663	0.324	8.543	0.609	0.542
	Petrovic, 2011	0.896	0.503	1.597	-0.372	0.710
	Kuyvenhoven, 1999	1.382	0.689	2.770	0.911	0.362
	Klaamas, 1997	1.200	0.507	2.840	0.415	0.678
	Yei, 2005	1.238	0.592	2.589	0.566	0.571
	Loffeld, 1991	0.746	0.492	1.129	-1.386	0.166
	Heneghan, 1998	0.921	0.524	1.617	-0.287	0.774
	deMattos, 2002	4.176	1.841	9.471	3.421	0.001
	Turkolmez, 2007	0.905	0.610	1.343	-0.496	0.620
	Robertson, 2003	0.940	0.645	1.370	-0.321	0.748
	Bhuiyan, 2009	0.771	0.448	1.325	-0.941	0.347
	Henrikkson, 1993	1.441	0.349	5.948	0.505	0.613
	Jafarzadeh, 2007	0.699	0.465	1.049	-1.731	0.084
	Kanbay, 2005	1.523	1.047	2.216	2.198	0.028
	Ansari, 2015	7.025	3.740	13.195	6.062	0.000
	Keller, 2002	1.191	0.770	1.843	0.787	0.431
	Bayan, 2008	0.912	0.604	1.378	-0.437	0.662
	Oberhuber, 1997	0.922	0.474	1.792	-0.240	0.810
	Wu, 2003	0.868	0.571	1.320	-0.662	0.508
	Tadesse, 2014	1.050	0.617	1.787	0.180	0.857
	Moges, 2006	0.854	0.397	1.838	-0.402	0.687
	Klaamas, 1999	0.885	0.526	1.490	-0.458	0.647
	Lin, 1998	9.904	6.778	14.472	11.849	0.000
	Nakao, 2011	1.115	0.883	1.407	0.915	0.360
	Risch, 2010	1.153	0.846	1.570	0.902	0.367
	Rizzato, 2013	1.000	0.834	1.200	0.003	0.998
	Hook-Nikanne, 1990	0.995	0.587	1.685	-0.020	0.984
Fixed		1.163	1.074	1.259	3.731	0.000
Random		1.230	0.984	1.539	1.818	0.069

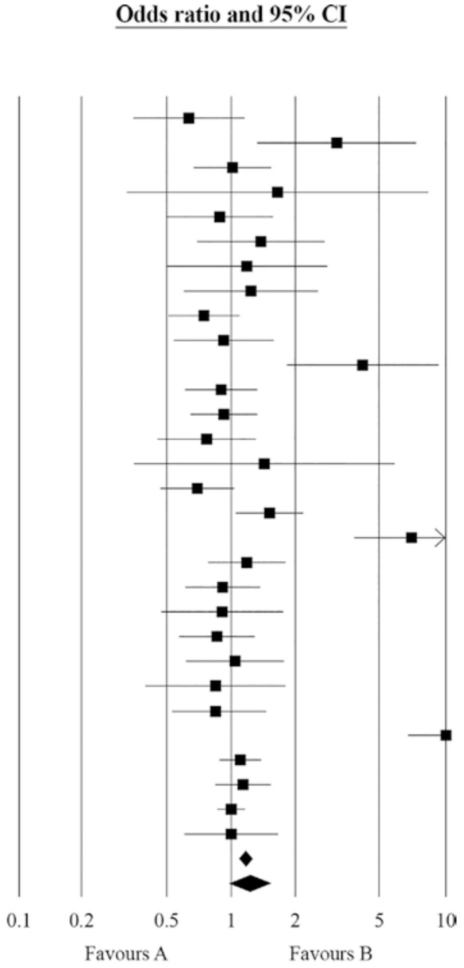

Fig. 27.1 Meta-analysis of the association between O blood group and *H. pylori* infection. For each study, the box represents the fixed- and random-effects odds ratio and the line represents the 95% confidence intervals. The size of each box indicates the relative weight of each study in the meta-analysis. Test for overall fixed and random effects: $z = 3.731$, $p < 0.001$, $I^2 = 85.52$, $p < 0.001$; $z = 1.818$, $p = 0.069$, respectively. (Adapted from Chakrani et al. [2])

Model	Study name	Statistics for each study				
		Odds ratio	Lower limit	Upper limit	Z-Value	*p*-Value
	Niv, 1996	0.926	0.400	2.144	-0.180	0.857
	Valliani, 2013	0.246	0.051	1.183	-1.750	0.080
	Umlauft, 1996	0.754	0.325	1.746	-0.659	0.510
	Rasmi, 2011	0.320	0.135	0.762	-2.576	0.010
	Petrovic, 2011	0.952	0.475	1.911	-0.137	0.891
	Klaamas, 1997	0.917	0.284	2.956	-0.146	0.884
	Yei, 2005	1.854	0.679	5.065	1.204	0.229
	Loffeld, 1991	1.133	0.549	2.338	0.339	0.735
	Heneghan, 1998	1.389	0.513	3.762	0.646	0.518
	deMattos, 2002	0.923	0.306	2.788	-0.142	0.887
	Turkolmez, 2007	0.783	0.449	1.367	-0.860	0.390
	Robertson, 2003	1.040	0.594	1.820	0.137	0.891
	Bhuiyan, 2009	0.901	0.511	1.586	-0.362	0.717
	Henrikkson, 1993	0.690	0.056	8.450	-0.291	0.771
	Jafarzadeh, 2007	1.233	0.783	1.943	0.905	0.365
	Kanbay, 2005	0.836	0.537	1.300	-0.795	0.427
	Ansari, 2015	0.429	0.218	0.844	-2.450	0.014
	Keller, 2002	0.978	0.443	2.160	-0.055	0.956
	Bayan, 2008	0.766	0.434	1.353	-0.918	0.359
	Oberhuber, 1997	1.308	0.455	3.755	0.499	0.618
	Wu, 2003	1.059	0.641	1.750	0.223	0.823
	Tadesse, 2014	0.794	0.449	1.401	-0.797	0.425
	Moges, 2006	1.357	0.573	3.213	0.694	0.488
	Klaamas, 1999	0.964	0.533	1.743	-0.122	0.903
	Lin, 1998	0.110	0.061	0.198	-7.390	0.000
	Nakao, 2011	0.890	0.691	1.147	-0.902	0.367
	Hook-Nikanne, 1990	1.108	0.575	2.138	0.307	0.759
Fixed		0.831	0.738	0.935	-3.073	0.002
Random		0.819	0.663	1.011	-1.858	0.063

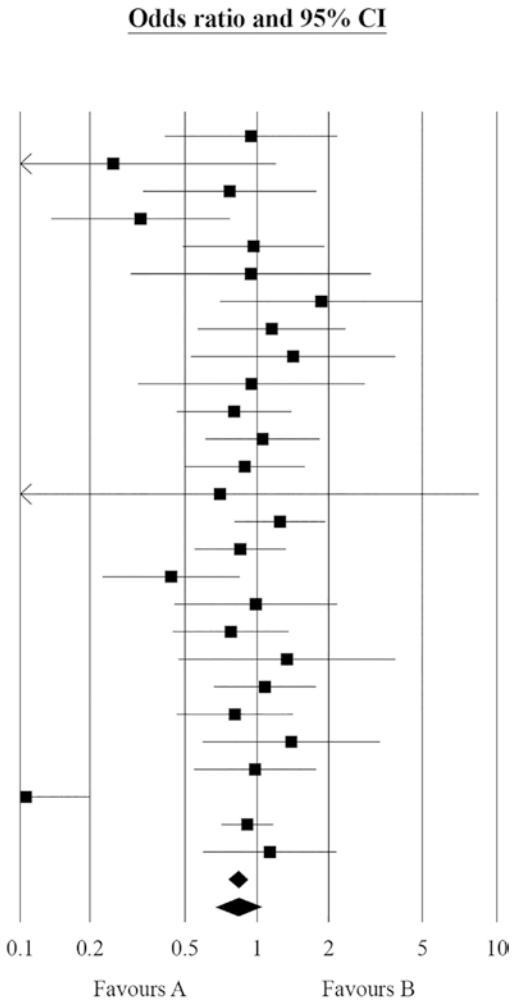

Fig. 27.2 Meta-analysis of the association between B blood group and *H. pylori* infection. Test for overall fixed and random effects: $z = -3.073$, $p = 0.002$, $I^2 = 62.31$, $p < 0.001$; $z = -1.858$, $p = 0.063$, respectively. (Adapted from Chakrani et al. [2])

Model	Study name	Statistics for each study				
		Odds ratio	Lower limit	Upper limit	Z-Value	*p*-Value
	Niv, 1996	6.811	0.865	53.613	1.822	0.068
	Valliani, 2013	0.514	0.051	5.144	-0.566	0.571
	Umlauft, 1996	1.099	0.271	4.466	0.132	0.895
	Rasmi,2011	1.399	0.703	2.786	0.956	0.339
	Petrovic, 2011	0.391	0.139	1.101	-1.778	0.075
	Klaamas, 1997	0.902	0.240	3.391	-0.153	0.879
	Yei, 2005	0.656	0.133	3.235	-0.518	0.605
	Loffeld, 1991	0.799	0.242	2.643	-0.367	0.714
	Heneghan, 1998	0.591	0.106	3.303	-0.599	0.549
	deMattos, 2002	0.930	0.149	5.785	-0.078	0.938
	Turkolmez, 2007	1.014	0.536	1.916	0.041	0.967
	Robertson, 2003	1.678	0.613	4.588	1.008	0.314
	Bhuiyan, 2009	0.578	0.254	1.311	-1.312	0.189
	Henrikkson, 1993	1.949	0.087	43.784	0.420	0.674
	Jafarzadeh, 2007	0.907	0.455	1.809	-0.276	0.782
	Kanbay, 2005	0.149	0.080	0.278	-5.994	0.000
	Ansari, 2015	0.505	0.236	1.079	-1.763	0.078
	Keller, 2002	1.465	0.528	4.059	0.734	0.463
	Bayan, 2008	0.491	0.194	1.247	-1.495	0.135
	Oberhuber, 1997	0.634	0.177	2.266	-0.701	0.483
	Wu, 2003	0.999	0.404	2.474	-0.001	0.999
	Tadesse, 2014	1.630	0.366	7.257	0.641	0.522
	Moges, 2006	0.659	0.133	3.260	-0.511	0.609
	Klaamas, 1999	1.771	0.609	5.154	1.049	0.294
	Lin, 1998	0.511	0.350	0.745	-3.488	0.000
	Nakao, 2011	0.832	0.581	1.190	-1.008	0.313
	Hook-Nikanne, 1990	0.755	0.321	1.776	-0.643	0.520
Fixed		0.709	0.605	0.832	-4.235	0.000
Random		0.767	0.594	0.991	-2.027	0.043

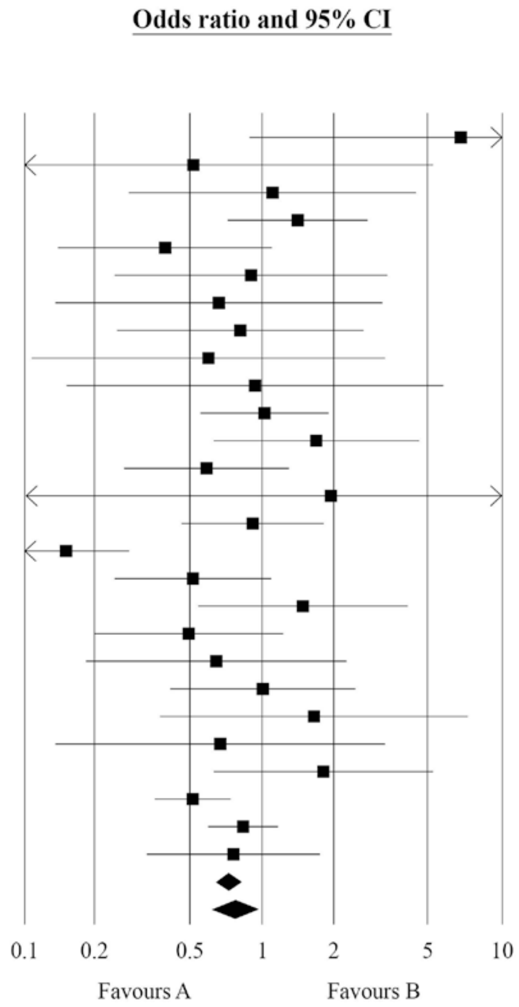

Fig. 27.3 Meta-analysis of the association between AB blood group and *H. pylori* infection. Test for overall fixed and random effects: $z = -4.235$, $p < 0.001$, $I^2 = 49.27$, $p = 0.002$; $z = -2.027$, $p = 0.043$, respectively. (Adapted from Chakrani et al. [2])

27.4 Research Studies Explaining the Interaction Between *H. pylori* and ABO Antigen, the Results, and Hypotheses

ABO antigens are glycolipids which are determined by a gene on chromosome 9q34.2 and expressed on cell membrane. Glycosyltransferases are coded in that gene. For blood type A, *N*-acetylgalactosamine is transferred on the H antigen, and for blood type B, D-galactose is transferred by the blood type-specific transferases. When the gene has a loss-of-function mutation, such glycosylation cannot occur, and the phenotype becomes blood type O. In case of blood type AB, the person has two glycosyltransferases and gets A and B antigens altogether [46] (Fig. 27.4). Additive glycosylation onto the already-formed ABO antigen can occur by Lewis (Le) enzyme, making Le antigen [21] (Fig. 27.5). These blood antigens are not only expressed on the red blood cells but also on various human cells including gastric mucosal cells.

27.4.1 Immune Evasion by Molecular Mimicry

Lipopolysaccharides (LPS) are expressed on the bacterial membrane of *H. pylori*. The O-chain of LPS has polysaccharides with structural similarity to human Le antigen. It is called molecular mimicry, and antigens such as Le^{x}, Le^{y}, Le^{a}, Le^{b}, Le^{c}, sialyl-Le^{x}, and H-1 and the related blood groups A and B are described. Le^{x} and Le^{y} are common properties expressed in as much as 80–90% of *H. pylori* strains screened serologically, but Le^{a} and Le^{b} are more common in Asian isolates. Moreover, a mosaicism in which Le antigen and blood group antigens are expressed in the same O-chain contributes to antigenic

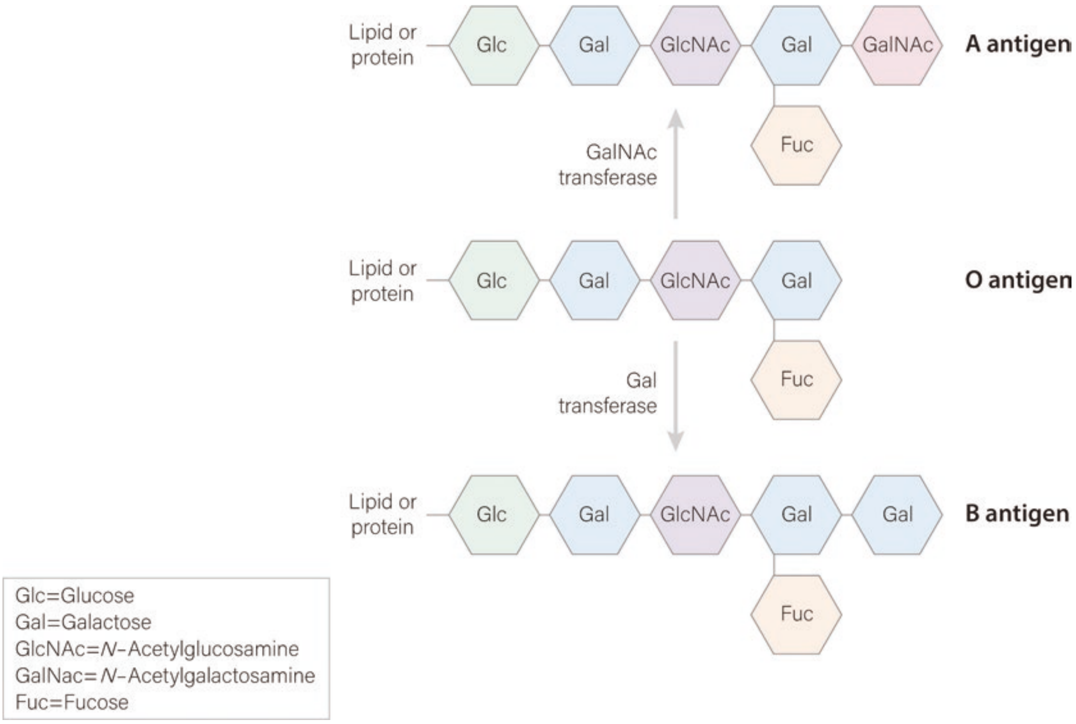
Lipid or protein
Glc
Gal
GlcNAc
Gal
GalNAc
A antigen
Fuc
GalNAc transferase
Lipid or protein
Glc
Gal
GlcNAc
Gal
O antigen
Fuc
Gal transferase
Lipid or protein
Glc
Gal
GlcNAc
Gal
Gal
B antigen
Fuc
Glc=Glucose
Gal=Galactose
GlcNAc=N-Acetylglucosamine
GalNac=N-Acetylgalactosamine
Fuc=Fucose

Fig. 27.4 Structures of ABO antigens. (Adapted from Lodish et al. [46])

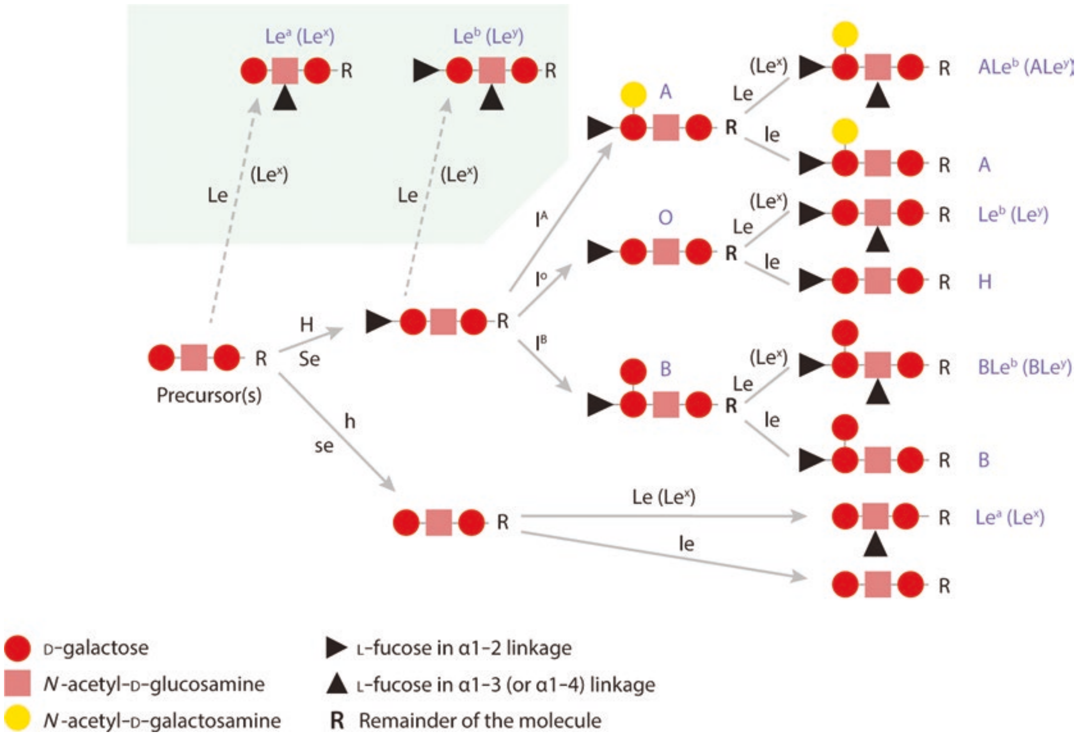
Le^a (Le^x)
Le^b (Le^y)
Le
(Le^x)
Le
(Le^x)
Precursor(s)
H
Se
h
se
I^A
I^O
I^B
A
O
B
Le
le
(Le^x)
ALe^b (ALe^y)
A
Le^b (Le^y)
H
BLe^b (BLe^y)
B
Le (Le^x)
le
Le^a (Le^x)
R
● D-galactose
■ N-acetyl-D-glucosamine
● N-acetyl-D-galactosamine
▶ L-fucose in α1-2 linkage
▲ L-fucose in α1-3 (or α1-4) linkage
R Remainder of the molecule

Fig. 27.5 ABO and Lewis antigens. (Adapted from Daniels [21])

diversity. Genetically modified *H. pylori* strains unable to express Le antigen, compared to the parental strains, failed to induce gastritis in animal studies, which support that Le antigen has an important role in disease development by *H. pylori*. This phenomenon can be explained by the fact that such molecular mimicry helps as a mechanism of immune evasion [47].

27.4.2 Adhesion Molecules onto Which *H. pylori* can Attach

Another explanation of the role of blood antigen in *H. pylori* infection is that the blood antigens are adhesion molecules onto which *H. pylori* attach. Thus, *H. pylori* can adhere to the "buttress" molecules on the surface of host mucosal membrane and induce chronic inflammation. For example, a protein BabA expressed on *H. pylori* is known to bind Le^b and related terminal fucose residues found on A, B, and H antigens expressed on gastric mucosal cells. However, not all *H. pylori* strains express BabA since there can be loss-of-function mutations, and there are strains producing BabA which do not bind Le^b and ABO antigens. The mutated BabA may bind to other unknown oligosaccharides distinct from Le^b and ABO antigens. Functional BabA is more commonly found in patients with duodenal ulcer and gastric adenocarcinoma than in patients with asymptomatic gastritis. Therefore, BabA is considered as an important virulent factor in the pathogenicity of *H. pylori*. This is consistent with the observation that peptic ulcer is more common in blood type O individuals, in whom H and Le^b are predominantly expressed on the gastric epithelium [48].

27.4.3 Decoy Mechanism by Secretors

However, there is secretor gene (Se), which is inherited independently from ABO blood group. The individuals with functional gene, secretors, have the ability to secrete α1,2-fucosylated glycan produced by *ABO* gene. However, nonsecretors cannot secrete the glycan but have the glycan expressed on the surface of cellular membrane. Secreted glycans are present in the mucous on the gastric epithelium and competitively bind to adhesive molecules on the bacterial membrane of *H. pylori*. As such, secreted glycans act as "decoys" with which the gastric mucosal cells can evade attachment by *H. pylori*. Therefore, nonsecretors lacking "decoy" seem to be more vulnerable to *H. pylori* infection and chronic inflammation [48].

27.5 The Association of ABO Blood Group with Gastric Cancer

Studies on ABO blood type should consider that *ABO* allele frequencies vary widely with ethnicity and geography. The association of ABO blood group with GC was first noticed in the 1950s. Later, a research conducted in 15 places in the USA, Europe, and Australia showed that blood type A increases the risk of GC compared with blood type O (OR 1.24; 95% CI, 1.18–1.30) [49]. In 2010, the result of a prospective study (Scandinavian Donations and Transfusions, SCANDAT) which followed Danish and Swedish blood donors of 1,089,022 during 35 years was published, where blood type A increased the risk of GC compared to blood type O (OR 1.20; 95% CI, 1.02–1.42) but the risk of peptic ulcer was higher in blood type O [50]. These two studies included only European descendant Caucasians. Interestingly, ABO alleles and the fucosyltransferase (FUT) enzymes and genes which are involved in Lewis antigen formation [and in *H. pylori* binding and pathogenicity] were suggested to be related to GC risk [3]. From this background, Mao et al. conducted an investigation of 32 variants at *ABO* and *FUT1–7* loci and GC risk in a case–control study of 365 cases and 1284 controls nested within the EPIC cohort (the EPIC-EurGast study) [3]. Four variants (including rs505922) in *ABO* and allelic blood group A (*AO* + *AA*, OR 51.84; 95% CI, 1.20–2.80) were associated with diffuse-type GC; however, conditional models with other *ABO* variants indicated that the associations were largely due to allelic blood group A [3]. One variant in *FUT5* was also associated with diffuse-type GC, and four variants (and haplotypes) in *FUT2* (Se), *FUT3* (Le), and *FUT6* were associated with intestinal-type GC [3]. Furthermore, one variant in *ABO*, two in *FUT3,* and two in *FUT6* were found to be associated with *H. pylori* infection status in controls, and two of these (in *FUT3* and *FUT6*) were weakly associated with intestinal-type GC risk [3]. None of the individual variants surpassed a Bonferroni-corrected *p*-value cutoff of 0.0016. However, after a gene-based permutation test, two loci [*FUT3*(Le)/*FUT5*/*FUT6* and *FUT2*(Se)] were significantly associated with diffuse- and intestinal-type GC, respectively [3]. Taken together, the role of *ABO* and *FUT* alleles in *H. pylori* infection is different depending on subtype-specific gastric carcinogenesis.

Recently, there were several studies with Asian population. A study conducted in Japan analyzed the association of ABO blood type with GC by *ABO* genotype. Genotypes *OO* and *BO*, compared to genotype *AA*, had decreased risk of GC by OR 0.70 (95% CI, 0.50–0.99) and OR 0.53 (95% CI, 0.36–0.77), respectively. Moreover, addition of A allele was associated with increased risk of GC ($p_{\text{trend}} < 0.001$), while addition of B allele was associated with decreased risk of GC ($p_{\text{trend}} = 0.023$) [51]. A study conducted in South Korea showed that genotypes *AA* and *AO*, compared to genotype *OO*, had increased risk of GC by OR 1.56 (95% CI, 1.08–2.26) and OR 1.57 (95% CI, 1.21–2.03), respectively. In subgroup analyses, statistically significant association was only observed in females with diffuse-type GC (OR 2.00; 95% CI, 1.43–2.78) [52]. These results suggest that research studies regarding ABO blood type should take into account because *ABO* allele frequencies vary widely upon ethnicity and geography. Results of several studies which probed the association of ABO blood type with cancer incidence [8–10] are compared in

Table 27.1 ABO genotype and allele frequency observed in cancer research studies

	Korean gastric cancer study [52]			Japanese gastric cancer study [51]			Chinese pancreatic cancer study [9]	US Caucasian breast cancer study [8]	North America pancreatic cancer study [10]
	Control ($n = 1700$)	Gastric cancer ($n = 3245$)	Total ($n = 4945$)	Control ($n = 1465$)	Gastric cancer ($n = 703$)	Total ($n = 2168$)	Total ($n = 1815$)	Total ($n = 3141$)	Total ($n = 6994$)
ABO genotype frequency, no. of subjects (%)									
AA	154 (9.1)	300 (9.2)	454 (9.2)	117 (8.0)	74 (10.5)	191 (8.8)	117 (6.4)	229 (7.3)	563 (8.0)
AO	494 (29.1)	1087 (33.5)	1581 (32.0)	455 (31.1)	245 (34.9)	700 (32.3)	479 (26.4)	1098 (35.0)	2492 (35.6)
BB	77 (4.5)	127 (3.9)	204 (4.1)	34 (2.3)	11 (1.6)	45 (2.1)	74 (4.1)	17 (0.5)	67 (1.0)
BO	337 (19.8)	555 (17.1)	892 (18.0)	290 (19.8)	98 (13.9)	388 (17.9)	416 (22.9)	304 (9.7)	751 (10.7)
AB	202 (11.9)	393 (12.1)	595 (12.0)	141 (9.6)	81 (11.5)	222 (10.2)	200 (11.0)	139 (4.4)	336 (4.8)
OO	436 (25.6)	783 (24.1)	1219 (24.7)	428 (29.2)	194 (27.6)	622 (28.7)	529 (29.1)	1354 (43.1)	2785 (39.8)
ABO allele frequency									
A	0.295	0.32	0.312	0.283	0.337	0.301	0.252	0.27	0.283
B	0.204	0.185	0.192	0.17	0.143	0.161	0.21	0.076	0.087
O	0.501	0.494	0.497	0.546	0.52	0.538	0.538	0.654	0.63

Allele frequencies were calculated according to the Hardy–Weinberg equilibrium

Table 27.1. The B allele frequency of European descendant Caucasian is half of that of Asian. This difference explains why the role of B allele in the development of GC was prominently noticed in studies done with Asians but not in those with Caucasian.

Recent meta-analysis validated the association of blood group A with risk of GC and suggested that blood group AB was also associated with GC risk [6]. In the case–control study, compared with blood group O, both blood groups A and AB were associated with increased GC risk (for group A, OR 1.13; 95% CI, 1.02–1.24; for group AB, OR 1.18; 95% CI, 1.02–1.36, respectively) [6]. In addition, analyses of *ABO* genotypes revealed associations of *AO* and *AB* with risk of GC compared with *OO* genotype [6]. Consistent with the case–control study, meta-analysis of 40 studies including 33,613 cases and 2,431,327 controls demonstrated that blood groups A (OR 1.19; 95% CI, 1.13–1.25) and AB (OR 1.09; 95% CI, 1.03–1.16) were associated with increased risk of GC [6]. For such findings to be accepted as a medical fact, there should be an explanation at the level of molecular biology. That is, functional investigations are warranted to elucidate the exact mechanism of ABO blood groups in gastric carcinogenesis. So far, there is no direct evidence which connects the ABO blood type and the development of GC. Only recently, ABO antigens were speculated to mediate the pathogenicity of *H. pylori* and, by doing so, increase the risk of GC.

27.6 The Association of ABO Blood Group with Gastric Cancer by Way of *H. pylori* Infection

H. pylori is the primary cause of GC, and its role in carcinogenesis is believed to be modulated by microbial, environmental, and host factors [53, 54]. It is clear that ABO blood type is associated with GC as a host factor. Blood group A is associated with increased risk of GC [50, 55], whereas the B allele is associated with decreased risk of GC [51]. Another study reported that such a relationship was sex- and histological type-specific [52]. In addition, various factors, such as low socioeconomic status (SES), smoking, family history of GC, and dietary factors, have been implicated in GC [56–65]. Moreover, distinctive etiologies exist for each subtype of GC according to location (cardia vs. non-cardia) and histological type (intestinal type and diffuse type). Not surprisingly, risk factors have different patterns and strengths of association with each subtype of GC. Current knowledge indicates that the carcinogenic mechanism might be affected by ABO blood antigens. Carriers of *cagA*-positive strains with blood type A have a higher risk of gastric precancerous lesions than those with blood type O [66]. However, there is a lack of evidence regarding how *H. pylori* infection status, eradication history, and ABO blood type affect the development of each subtype of GC when the multifactorial and distinctive carcinogenic etiology is fully considered.

From this background, our team performed a case-control study of 997 patients with non-cardia gastric cancer (NCGC) and 1147 control subjects using risk analyses with 14 factors including *H. pylori* infection with eradication history and *ABO* genotype [36] (Table 27.2). Multivariate logistic regression models showed that *ABO* genotype with the B allele was associated with a significantly lower risk of NCGC of both histological types [36] (Table 27.3). BB showed more risk reduction effect of NCGC (OR 0.54) than BX (OR 0.73) [36] (Table 27.3). The reduction in risk for NCGC by adding the B allele was more prominent in diffuse type than that in the intestinal type [36] (Table 27.3). In addition, the *ABO* genotype without the B allele revealed a stronger association with diffuse-type NCGC than with intestinal-type NCGC [36]. This result is consistent with the result of a Japanese study [51]. Western studies mainly show that the A allele increases the risk of GC, and the reason why the role of the B allele is more pronounced in Asian studies can be attributed to the fact that B allele frequency in European-descended Caucasians is significantly lower than that of Asians [36]. This role of ABO blood antigens in gastric carcinogenesis has been believed to be related to *H. pylori* infection. ABO

Table 27.2 Baseline characteristics of the control group and patients with non-cardia gastric cancer (NCGC)

	Control (*n* = 1147)	NCGC (*n* = 997)	*p*-value
Male	560 (48.8)	658 (66.0)	<0.001
Age (year, mean ± SD)	54.3 ± 14.2	59.9 ± 12.0	<0.001
<40	193 (15.8)	58 (5.8)	<0.001
40–59	500 (43.6)	384 (38.5)	
≥60	454 (39.6)	555 (55.7)	
BMI (kg/m^2, mean ± SD)	23.0 ± 3.3	23.2 ± 3.2	0.341
<23	577 (51.4)	467 (47.1)	0.156
≥23, <25	267 (23.8)	267 (26.9)	
≥25, <30	251 (22.4)	238 (24.0)	
≥30	27 (2.4)	19 (1.9)	
Number of 1° family members with gastric cancer			
Zero	1006 (88.5)	790 (80.0)	<0.001
One	122 (10.7)	174 (17.6)	
Two or more	9 (0.8)	23 (2.3)	
Childhood residency			
Urban area	632 (58.4)	379 (40.1)	<0.001
Rural area	451 (41.6)	567 (59.9)	
Socioeconomic status defined by final education or monthly income			
College or >$5000	609 (56.9)	357 (37.8)	<0.001
High school or $3000–5000	281 (26.3)	295 (31.2)	
Middle/elementary school and ≤$3000	180 (16.8)	293 (31.0)	
Smoking status			
Never smoker	659 (57.8)	387 (39.0)	<0.001
Ever smoker	482 (42.2)	605 (61.0)	
Alcohol intake			
Never drinker	382 (33.5)	284 (28.7)	0.016
Ever drinker	758 (66.5)	707 (71.3)	
Spicy diet			
Not/moderately spicy	799 (74.3)	653 (69.2)	0.010
Strongly spicy	276 (25.7)	291 (30.8)	
Salty diet			
Not/moderately salty	684 (62.7)	521 (54.7)	<0.001
Very salty	407 (37.3)	431 (45.3)	
Intake of processed meat or canned food			
Rarely	832 (78.2)	749 (81.0)	0.126
More than three times/week	232 (21.8)	176 (19.0)	
Intake of fruits or vegetables			
Not everyday	434 (40.0)	466 (49.3)	<0.001
Everyday	650 (60.0)	479 (50.7)	
ABO genotype			
AA	82 (7.1)	78 (7.8)	0.038
AO	331 (28.9)	313 (31.4)	
BB	66 (5.8)	37 (3.7)	
BO	264 (23.0)	190 (19.1)	
AB	121 (10.5)	104 (10.4)	
OO	283 (24.7)	275 (27.6)	

Table 27.2 (continued)

	Control (*n* = 1147)	NCGC (*n* = 997)	*p*-value
H. pylori infection status incorporated with history of eradication			
The uninfected	322 (28.6)	143 (14.4)	<0.001
Past infection, eradication history (+)	156 (13.9)	79 (8.0)	
Current infection, eradication history (+)	120 (10.7)	37 (3.7)	
Past infection, eradication history (−)	90 (8.0)	126 (12.7)	
Current infection, eradication history (−)	436 (38.8)	607 (61.2)	

Values are numbers (%)
(Adapted from Oh et al. [36])
NCGC non-cardiac gastric cancer, *SD* standard deviation, *BMI* body mass index, 1° first degree

Table 27.3 Multivariable logistic regression analysis of risk factors for non-cardia gastric cancer (NCGC) and intestinal and diffuse types of NCGC

	NCGC (*n* = 898), control (*n* = 999)		Intestinal-type NCGC (*n* = 493), control (*n* = 981)		Diffuse-type NCGC (*n* = 359), control (*n* = 1011)	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Sex						
Female	1		1		1	
Male	1.22 (0.90–1.66)	0.208	2.07 (1.40–3.08)	<0.001	0.72 (0.48–1.08)	0.109
Age						
<40	1		1		1	
40–59	2.00 (1.37–2.91)	<0.001	6.94 (2.91–16.6)	<0.001	1.37 (0.90–2.07)	0.140
≥60	2.98 (2.03–4.37)	<0.001	17.2 (7.22–41.2)	<0.001	1.14 (0.74–1.77)	0.555
BMI group						
<23	1		1			
≥23, <25	1.03 (0.80–1.31)	0.830	1.37 (1.02–1.86)	0.039		
≥25, <30	0.99 (0.77–1.28)	0.939	1.17 (0.85–1.60)	0.344		
≥30	0.99 (0.49–1.97)	0.970	1.31 (0.54–3.19)	0.550		
Number of 1° family members with gastric cancer						
Zero	1		1		1	
One	1.50 (1.12–2.00)	0.006	1.56 (1.10–2.21)	0.012	1.52 (1.04–2.21)	0.029
At least two	3.64 (1.45–9.11)	0.006	3.38 (1.20–9.53)	0.021	2.38 (0.77–7.35)	0.131
Childhood residency						
Urban area	1		1		1	
Rural area	1.50 (1.21–1.84)	<0.001	1.64 (1.26–2.14)	<0.001	1.24 (0.94–1.63)	0.127

(continued)

	NCGC ($n = 898$), control ($n = 999$)		Intestinal-type NCGC ($n = 493$), control ($n = 981$)		Diffuse-type NCGC ($n = 359$), control ($n = 1011$)	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Socioeconomic status defined by final education or monthly income						
College or >$5000	1		1		1	
High school or $3000–5000	1.48 (1.16–1.88)	0.001	1.64 (1.21–2.22)	0.001	1.32 (0.97–1.81)	0.080
Middle/elementary school and ≤$3000	1.78 (1.35–2.34)	<0.001	2.25 (1.61–3.15)	<0.001	1.47 (1.02–2.12)	0.039
Smoking status						
Never smoker	1		1		1	
Ever smoker	1.65 (1.22–2.23)	0.001	1.42 (0.97–2.06)	0.069	1.76 (1.18–2.62)	0.005
Alcohol intake						
Never drinker	1		1			
Ever drinker	0.97 (0.76–1.24)	0.794	1.08 (0.79–1.48)	0.634		
Spicy diet						
Not/moderately spicy	1		1		1	
Strongly spicy	1.05 (0.83–1.34)	0.678	0.92 (0.67–1.24)	0.570	1.13 (0.85–1.51)	0.411
Salty diet						
Not/moderately salty	1		1			
Very salty	1.12 (0.90–1.40)	0.316	1.36 (1.04–1.80)	0.028		
Intake of processed meat or canned food						
Rarely			1			
More than three times/week			1.28 (0.91–1.82)	0.162		
Intake of fruits or vegetables						
Not every day intake	1		1		1	
Everyday intake	0.78 (0.64–0.96)	0.018	1.20 (0.93–1.55)	0.169	0.069 (0.53–0.90)	0.006
ABO genotype in accordance with number of B allele						
XX	1		1		1	
BX	0.73 (0.59–0.91)	0.005	0.72 (0.55–0.95)	0.018	0.64 (0.48–0.86)	0.004
BB	0.54 (0.33–0.86)	0.009	0.59 (0.32–1.06)	0.079	0.39 (0.20–0.76)	0.006

Table 27.3 (continued)

	NCGC (n = 898), control (n = 999)		Intestinal-type NCGC (n = 493), control (n = 981)		Diffuse-type NCGC (n = 359), control (n = 1011)	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
H. pylori infection status incorporated with history of eradication						
Current infection, eradication history (−)	1		1		1	
Past infection, eradication history (−)	0.95 (0.68–1.33)	0.760	1.06 (0.69–1.61)	0.798	0.84 (0.56–1.28)	0.421
Current infection, eradication history (+)	0.22 (0.14–0.34)	<0.001	0.34 (0.20–0.59)	<0.001	0.11 (0.05–0.24)	<0.001
Past infection, eradication history (+)	0.44 (0.32–0.61)	<0.001	0.56 (0.37–0.84)	0.005	0.29 (0.18–0.47)	<0.001
The uninfected	0.31 (0.24–0.40)	<0.001	0.38 (0.27–0.53)	<0.001	0.22 (0.15–0.32)	<0.001
Hosmer–Lemeshow goodness-of-fit test, *p*-value	0.99	0.185		0.648		

(Adapted from Oh et al. [36])
NCGC non-cardiac gastric cancer, 1° first degree, *X* either the A or O allele

antigens are expressed on various cells, including gastric mucosal cells. Lewis antigen b (Le^b) and related terminal fucose residues on ABO antigens serve as a buttress onto which *H. pylori* attaches and causes chronic inflammation by interacting with the BabA protein expressed on the *H. pylori* bacterial membrane [47, 48, 67]. However, in our analyses, *ABO* genotype and *H. pylori* infection status did not reveal any interaction but were independent risk factors. The associations between *ABO* genotype and *H. pylori* infection, atrophic gastritis, and intestinal metaplasia were not sufficiently evident in our separate analyses with the control population [36]. Thus, whether ABO blood antigens affect gastric carcinogenesis through interactions with *H. pylori* or by another mechanism unrelated to *H. pylori* remains uncertain and deserves future investigation.

27.7 Conclusions

Systemic reviews and meta-analysis showed the association of blood group A with risk of GC and suggested that blood group AB was also associated with GC risk. That is, evidences which support that blood group antigens are playing important roles in the development of GC have been observed. Probably, this association between ABO alleles and GC could be linked to the pathogenicity of *H. pylori*. Most of the research studies have been conducted at the level of animal studies and molecular biological experiments. Recently, these findings are frequently observed in human studies in European and Asian cohorts. However, there has not been a study which investigated the direct interaction between ABO blood group antigens and *H. pylori*. Thus, functional investigations are warranted to elucidate the exact mechanism of ABO blood groups in gastric carcinogenesis in which *H. pylori* plays an important role.

References

1. Abegaz SB. Human ABO blood groups and their associations with different diseases. Biomed Res Int. 2021;2021:6629060.
2. Chakrani Z, Robinson K, Taye B. Association between ABO blood groups and *Helicobacter pylori* infection: a meta-analysis. Sci Rep. 2018;8:17604.
3. Duell EJ, Bonet C, Munoz X, Lujan-Barroso L, Elisabete Weiderpass E, Boutron-Ruault MC, et al. Variation at ABO histo-blood group and FUT loci and diffuse and intestinal gastric cancer risk in a European population. Int J Cancer. 2015;136:880–93.
4. Xu YQ, Jiang TW, Cui YH, Zhao YL, Qiu LQ. Prognostic value of ABO blood group in patients with gastric cancer. J Surg Res. 2016;201:188–95.
5. Yu H, Xu N, Li ZK, Xia H, Ren HT, Li N, et al. Association of ABO blood groups and risk of gastric cancer. Scand J Surg. 2020;109:309–13.
6. Mao Y, Yang W, Qi Q, Yu F, Wang T, Zhang H, et al. Blood groups A and AB are associated with increased gastric cancer risk: evidence from a large genetic study and systematic review. BMC Cancer. 2019;19:164.
7. Chen Y, Hu N, Liao L, Yu K, Shu XO, Zheng W, et al. ABO genotypes and the risk of esophageal and gastric cancers. BMC Cancer. 2021;21:589.
8. Gates MA, Xu M, Chen WY, Kraft P, Hankinson SE, Wolpin BM. ABO blood group and breast cancer incidence and survival. Int J Cancer. 2012;130:2129–37.
9. Risch HA, Yu H, Lu L, Kidd MS. ABO blood group, *Helicobacter pylori* seropositivity, and risk of pancreatic cancer: a case-control study. J Natl Cancer Inst. 2010;102:502–5.
10. Klein AP, Lindström S, Mendelsohn JB, Steplowski E, Arslan AA, Bueno-de-Mesquita HB, et al. An absolute risk model to identify individuals at elevated risk for pancreatic cancer in the general population. PLoS One. 2013;8:e72311.
11. Bulajic M, Panic N, Löhr JM. *Helicobacter pylori* and pancreatic diseases. World J Gastrointest Pathophysiol. 2014;5:380–3.
12. Li P, Shu Y, Gu Y. The potential role of bacteria in pancreatic cancer: a systematic review. Carcinogenesis. 2020;41:397–404.
13. Goldman L, Ausiello D. Cecil textbook of internal medicine. 24th ed. Philadelphia: Saunders; 2012.
14. Harvard School of Public Health. Disease Risk Index. 2008. http://www.diseaseriskindex.harvard.edu/update/hccpquiz.pl?lang=english&func=show&quiz=stomach&page=risk_list. Accessed 9 Feb 2015.
15. Colditz GA, Atwood KA, Emmons K, Monson RR, Willett WC, Trichopoulos D, et al. Harvard report on cancer prevention volume 4: Harvard cancer risk index. Cancer Causes Control. 2000;11:477–88.
16. Denomme GA. Molecular basis of blood group expression. Transfus Apher Sci. 2011;44:53–63.
17. Aliyu AA. Distribution of ABO and Rh (D) blood groups and associated traits: a study of the College of Nursing and Midwifery. Msc Thesis Dissertation, Kogi State, Obangede, 2016.
18. Sandler SG, Mallory D. Biological functions of blood groups in health and disease. Haematologia (Budap). 1995;27:1–13.
19. Green C. The ABO, Lewis and related blood group antigens; a review of structure and biosynthesis. FEMS Microbiol Immunol. 1989;1:321–30.
20. Ewald DR, Sumner SC. Blood type biochemistry and human disease. Wiley Interdiscip Rev Syst Biol Med. 2016;8:517–35.
21. Daniels G. Human blood groups. 3rd ed. Oxford: Wiley-Blackwell; 2013.
22. Cartron JP, Colin Y. Structural and functional diversity of blood group antigens. Transfus Clin Biol. 2001;8:163–99.
23. Telen MJ. Erythrocyte adhesion receptors: blood group antigens and related molecules. Transfus Med Rev. 2005;19:32–44.
24. Spring FA, Parsons SF. Erythroid cell adhesion molecules. Transfus Med Rev. 2000;14:351–63.
25. Than NG, Romero R, Meiri H, Erez O, Xu Y, Tarquini F, et al. PP13, maternal ABO blood groups and the risk assessment of pregnancy complications. PLoS One. 2011;6:e21564.
26. Naeini AE, Rostami M, Naeini SE. Chronic viral hepatitis and their relation to ABO blood groups and rhesus (Rh) factor. Med Case Stud. 2010;1:5–7.
27. Jefferys SD, Kenneth CA. Chapter 109. Transfusion biology and therapy. In: Kasper DL, Braunwald E, Hauser S, Longo D, Jameson JL, Fauci AS, editors. Harrison's principles of internal medicine. 16th ed. New York: McGraw Hill; 2005.
28. Odeigah PG. Influence of blood group and secretor genes on susceptibility to duodenal ulcer. East Afr Med J. 1990;67:487–500.
29. Daniels G. Blood group polymorphisms: molecular approach and biological significance. Transfus Clin Biol. 1997;4:383–90.
30. Calafell F, Roubinet F, Ramírez-Soriano A, Saitou N, Bertranpetit J, Blancher A. Evolutionary dynamics of the human ABO gene. Hum Genet. 2008;124:123–35.
31. Megraud F, Brassens-Rabbe MP, Denis F, Belbouri A, Hoa DQ. Seroepidemiology of *Campylobacter pylori* infection in various populations. J Clin Microbiol. 1989;27:1870–3.
32. Go MF. Review article: Natural history and epidemiology of *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2002;16 (Suppl 1):3–15.

33. Safavi M, Sabourian R, Foroumadi A. Treatment of *Helicobacter pylori* infection: current and future insights. World J Clin Cases. 2016;4:5–19.
34. Rossez Y, Maes E, Lefebvre Darroman T, Gosset P, Ecobichon C, Joncquel Chevalier Curt M, et al. Almost all human gastric mucin O-glycans harbor blood group A, B or H antigens and are potential binding sites for *Helicobacter pylori*. Glycobiology. 2012;22:1193–206.
35. Clarke CA, Edwards JW, Haddock DR, Howel-Evans AW, Mcconnell RB, Sheppard PM. ABO blood groups and secretor character in duodenal ulcer; population and sibship studies. Br Med J. 1956;2:725–31.
36. Oh S, Kim N, Kwon JW, Shin CM, Choi YJ, Lee DH, et al. Effect of *Helicobacter pylori* eradication and ABO genotype on gastric cancer development. Helicobacter. 2016;21:596–605.
37. Cooling L. Blood groups in infection and host susceptibility. Clin Microbiol Rev. 2015;28:801–70.
38. Borén T, Normark S, Falk P. *Helicobacter pylori*: molecular basis for host recognition and bacterial adherence. Trends Microbiol. 1994;2:221–8.
39. Borén T, Falk P, Roth KA, Larson G, Normark S. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science. 1993;262:1892–5.
40. Kanbay M, Gür G, Arslan H, Yilmaz U, Boyacioglu S. The relationship of ABO blood group, age, gender, smoking, and *Helicobacter pylori* infection. Dig Dis Sci. 2005;50:1214–7.
41. Niv Y, Fraser G, Delpre G, Neeman A, Leiser A, Samra Z, et al. *Helicobacter pylori* infection and blood groups. Am J Gastroenterol. 1996;91:101–4.
42. Loffeld RJ, Stobberingh E. *Helicobacter pylori* and ABO blood groups. J Clin Pathol. 1991;44:516–7.
43. Henriksson K, Uribe A, Sandstedt B, Nord CE. *Helicobacter pylori* infection, ABO blood group, and effect of misoprostol on gastroduodenal mucosa in NSAID-treated patients with rheumatoid arthritis. Dig Dis Sci. 1994;38:1688–96.
44. Dickey W, Collins JS, Watson RG, Sloan JM, Porter KG. Secretor status and *Helicobacter pylori* infection are independent risk factors for gastroduodenal disease. Gut. 1993;34:351–3.
45. Ayalew F, Tilahun B, Taye B. Performance evaluation of laboratory professionals on malaria microscopy in Hawassa Town, Southern Ethiopia. BMC Res Notes. 2014;7:839.
46. Lodish H, Berk A, Kaiser CA, Krieger M. Molecular cell biology. 7th ed. New York: W.H. Freeman and Company; 2012.
47. Moran AP. Lipopolysaccharide in bacterial chronic infection: insights from *Helicobacter pylori* lipopolysaccharide and lipid A. Int J Med Microbiol. 2007;297:307–19.
48. Moore ME, Borén T, Solnick JV. Life at the margins: modulation of attachment proteins in *Helicobacter pylori*. Gut Microbes. 2011;2:42–6.
49. Clarke CA. Blood group and disease. In: Steiberg AG, editor. Progress in medical genetics, vol. 1. New York: Grunne and Stratton; 1961. p. 81–119.
50. Edgren G, Hjalgrim H, Rostgaard K, Norda R, Wikman A, Melbye M, et al. Risk of gastric cancer and peptic ulcers in relation to ABO blood type: a cohort study. Am J Epidemiol. 2010;172:1280–5.
51. Nakao M, Matsuo K, Ito H, Shitara K, Hosono S, Watanabe M, et al. ABO genotype and the risk of gastric cancer, atrophic gastritis, and *Helicobacter pylori* infection. Cancer Epidemiol Biomark Prev. 2011;20:1665–72.
52. Song HR, Shin MH, Kim HN, Piao JM, Choi JS, Hwang JE, et al. Sex-specific differences in the association between ABO genotype and gastric cancer risk in a Korean population. Gastric Cancer. 2013;16:254–60.
53. International Agency for Research on Cancer, World Health Organization. Cancer schistosomes, liver flukes and *Helicobacter pylori*. IARC Lyon Monogr Eval Carcinog Risks Hum. 1994;61:1–241.
54. Correa P. Gastric cancer: overview. Gastroenterol Clin N Am. 2013;42:211–7.
55. Wang Z, Liu L, Ji J, Zhang J, Yan M, Zhang J, et al. ABO blood group system and gastric cancer: a case-control study and meta-analysis. Int J Mol Sci. 2012;13:13308–21.
56. Shin CM, Kim N, Lee HS, Lee DH, Kim JS, Jung HC, et al. Intrafamilial aggregation of gastric cancer: a comprehensive approach including environmental factors, *Helicobacter pylori* virulence, and genetic susceptibility. Eur J Gastroenterol Hepatol. 2011;23:411–7.
57. Shin CM, Kim N, Yang HJ, Cho SI, Lee HS, Kim JS, et al. Stomach cancer risk in gastric cancer relatives: interaction between *Helicobacter pylori* infection and family history of gastric cancer for the risk of stomach cancer. J Clin Gastroenterol. 2010;44:e34–9.
58. Barker D, Coggon D, Osmond C, Wickham C. Poor housing in childhood and high rates of stomach cancer in England and Wales. Br J Cancer. 1990;61:575.
59. Roder DM. The epidemiology of gastric cancer. Gastric Cancer. 2002;5:5–11.
60. Ladeiras-Lopes R, Pereira AK, Nogueira A, Pinheiro-Torres T, Pinto I, Santos-Pereira R, et al. Smoking and gastric cancer: systematic review and meta-analysis of cohort studies. Cancer Causes Control. 2008;19:689–701.
61. La Torre G, Chiaradia G, Gianfagna F, De Lauretis A, Boccia S, Mannocci A, et al. Smoking status and gastric cancer risk: an updated meta-analysis of case-control studies published in the past 10 years. Tumori. 2009;95:13.
62. de Martel C, Forman D, Plummer M. Gastric cancer: epidemiology and risk factors. Gastroenterol Clin N Am. 2013;42:219–40.
63. Yan S, Li B, Bai ZZ, Wu JQ, Xie DW, Ma YC, et al. Clinical epidemiology of gastric cancer in

Hehuang valley of China: a 10-year epidemiological study of gastric cancer. World J Gastroenterol. 2014;20:10486–94.

64. Dikshit RP, Mathur G, Mhatre S, Yeole B. Epidemiological review of gastric cancer in India. Indian J Med Paediatr Oncol. 2011;32:3–11.
65. Yoon H, Kim N. Diagnosis and management of high risk group for gastric cancer. Gut Liver. 2015;9:5–17.
66. Rizzato C, Kato I, Plummer M, Munoz N, Stein A, Jan van Doorn L, et al. Risk of advanced gastric precancerous lesions in *Helicobacter pylori* infected subjects is influenced by ABO blood group and *cagA* status. Int J Cancer. 2013;133:315–22.
67. Eaton K, Logan S, Baker P, Peterson R, Monteiro M, Altman E. *Helicobacter pylori* with a truncated lipopolysaccharide O chain fails to induce gastritis in SCID mice injected with splenocytes from wild-type C57BL/6J mice. Infect Immun. 2004;72:3925–31.

28

Gastric Cancer: First Relatives of Gastric Cancer

Nayoung Kim and Yoon Jin Choi

Abstract

Family history is an important risk factor for gastric cancer (GC) and can be seen from 10 to 15% of GC patients. This factor is partially explained by genetic and environmental factors, and family history is also related to familial clustering of *Helicobacter pylori* (*H. pylori*) infection. This factor is notable because an immediate family history of GC itself increases GC genesis by two to three times, and this genesis increases by 5.88 times if *H. pylori* infection is also present. Along with a family history as a risk factor, some GC types with high genetic dependence can be classified as germline *CDHI* mutation-related hereditary diffuse-type GC, which was found to be 0.3–3.1% in Korea and Japan. Although family history increases GC genesis, this factor has been known to be beneficial for cancer prognosis. These subjects do frequent health screenings because of their immediate family history and follow good health-related behaviors, and the patients often show microsatellite instability (MSI) and mismatch repair proteins, which are related to a good prognosis. However, this issue is controversial in terms of overall survival. *H. pylori* eradication is the most important priority for first-degree family members of GC patients, who are less than 40s, especially those in their 20s and 30s when the intestinal metaplasia begins. Furthermore, as *H. pylori* eradication treatment reduces the risk of GC most guidelines strongly recommend *H. pylori* eradication in the first-degree relatives of GC with *H. pylori* infection.

Keywords

Gastric cancer · Family history · *Helicobacter pylori* · Eradication

28.1 Introduction

Gastric cancer (GC) is currently the fifth most common cancer, with approximately one million new cases diagnosed worldwide each year [1], and ranks as the fourth leading cause of cancer-related death [2]. It represents a particularly important public health issue in East Asian countries, where incidence rates are among the highest observed [3]. The age-standardized incidence rate of GC has been continually decreasing for a few decades in Japan [4] and Korea [5], but the number of new cases still exhibits an increasing

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pattern that is thought to be mainly attributable to the aging of the population. GC is induced by multifactorial interactions that include genetic and environmental aspects [6, 7] and *Helicobacter pylori* (*H. pylori*) infection. Then, the importance of family history has been proposed by many researchers as one of the GC risk factors [8, 9]. A famous example of GC pedigree would be Napoleon Bonaparte's family, since Napoleon himself and his family members died with GC. However, this category was classified as germline *CDHI* mutation-related hereditary diffuse gastric cancer (HDGC), and this rate was found to be 0.3–3.1% in Korea and Japan [10]. Usually, GC family history can be seen from 10 to 15% of GC patients, excluding familial GC patients, and gastric cancer relatives (GCRs) show two to three times higher possibility of getting GC compared with a normal control group [11–13]. Family history of GC has been explained along with genetic inheritance and environmental conditions that a family shares, such as dietary habit and smoking, but the relationship of familial clustering of *H. pylori* infection among family members has been added recently [14, 15]. That is, members of the same family tend to have similar environmental factors such as socioeconomic status and dietary habit, and there is a possibility that the same strain of *H. pylori* infection is also clustered. Therefore, the results of studies conducted to elucidate whether family history of GC is/is not an independent risk factor for GC should be interpreted very cautiously [16]. A population-based, statewide, case-control study from Germany reported that *H. pylori* infection and family history were strong independent risk factors for GC, although both were positively related to one another [17]. A Korean study also showed that the adjusted odds ratio (OR) for GC increased threefold for subjects with first-degree GCR (OR 2.85 in comparison with control; 95% confidential interval [CI], 1.83–4.46) [18]. Those patients had five to eight times higher possibility of getting GC than a normal control group [17, 18], which suggests that familial aggregation of GC could be explained at least partly by familial clustering of *H. pylori* infection [19]. Based on these GC research studies, the Maastricht guideline recommends *H. pylori* eradication therapy, if a patient with *H. pylori* infection has a family history of GC [20], and the *H. pylori* treatment guideline of Korea and Asia Pacific also recommends the same procedure [21, 22]. Therefore, this chapter is going to introduce how high the possibility of getting *H. pylori* infection if a patient has an immediate family history compared with a patient without a family history and the reason for GC genesis increment when both *H. pylori* infection and immediate family history on GC are present in a patient. Also, any difference in histological alterations between a *H. pylori*-infected patient with an immediate family history and a *H. pylori*-infected patient without the immediate family history, prognosis after GC diagnosis, and GC prevention method for patients with immediate family history on GC are going to be discussed.

28.2 Difference of *H. pylori* Infection Rate Depending on Family History on Gastric Cancer

The current *H. pylori* infection rate keeps decreasing in the world. For example, *H. pylori* seroprevalence among Koreans over 16 years old in 2005 and 2011 was 59.6% and 54.4%, respectively [23, 24], which significantly decreased from 66.9% in 1998 [25]. More dramatically, the prevalence decreased from 41.7 [26] to 23.5% [27] in the Czech Republic between 2001 and 2011. Based on a report from China [28] and Germany [19], *H. pylori* infection rate was higher in the GCRs than in a control group without a family history, and a similar result was also reported in the Korean report [13]. However, a nationwide epidemiological survey based on 10,796 health check-ups in Koreans in 2011 reported that *H. pylori* antibody-positive rate in the GCRs was 55.1%, while the rate for the healthy subjects without a family history was 54.4%, which did not show a difference [24], and this result was similar to the results from the surveys in 1998 [25] and in 2005 [23]. Thus, it can be inferred that a simple difference in *H. pylori* infection rate would not influence on GC rate of first-degree family members of GC patients. Thus, comprehensive research is needed in the big population of the world.

28.3 Risk Increment of Gastric Cancer in the Presence of *H. pylori* Infection and Family History of Gastric Cancer and the Preventive Effect of *H. pylori* Eradication

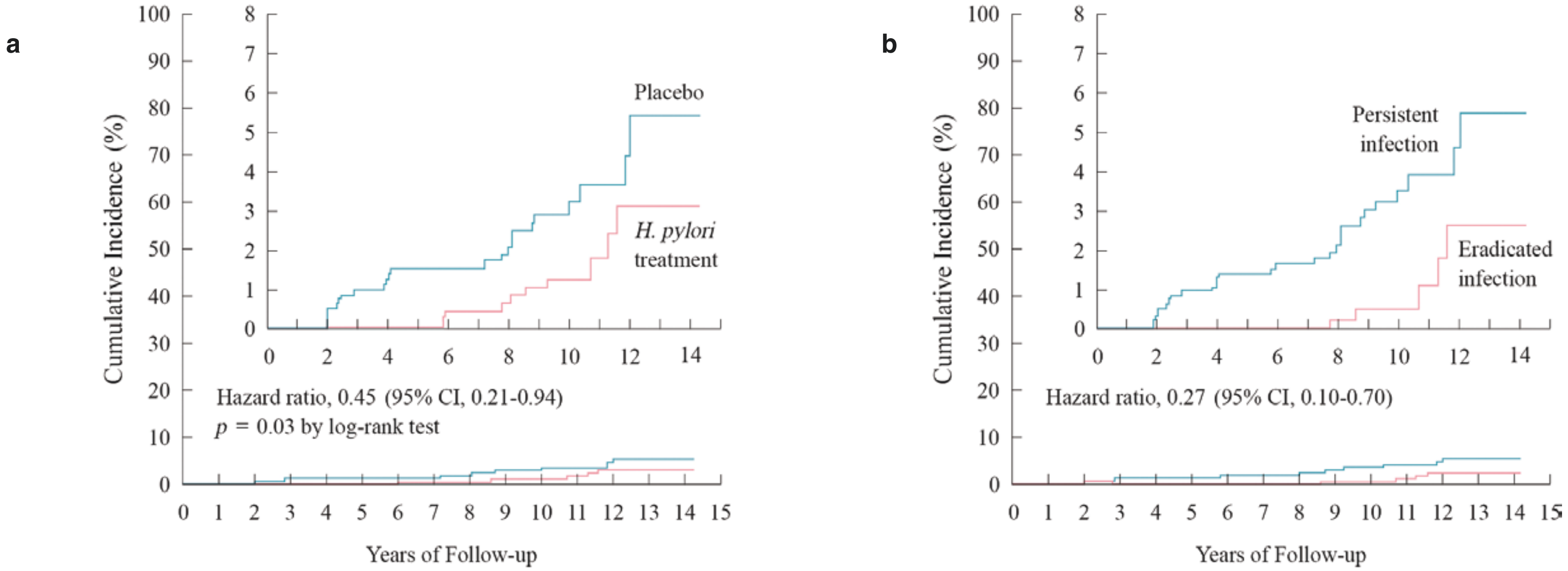
28.3.1 Risk Increment of Gastric Cancer in the Presence of *H. pylori* Infection and Family History of Gastric Cancer

The percentage of health check-up subjects who had a family history of GC was found to be 11% [24] or 11.8% [29], and a family history of GC showed two to three times higher possibility of getting GC patients than normal control groups, suggesting that the family history of GC is an independent risk factor [11–13]. Furthermore, first-degree relatives of patients with early-onset gastric carcinoma showed, even at younger ages, a high prevalence of *H. pylori* infection, high-risk operative link of gastritis assessment (OLGA), operative link for gastric intestinal metaplasia (OLGIM) stages and dysplasia [30]. This risk factor is partially explained by genetic aspect and environmental aspects (smoking, overconsumption of salt or *N*-nitroso compound, and antioxidant deficiency) [13], and familial clustering of *H. pylori* infection is also related [14, 15]. In particular, there was a synergistic effect if both *H. pylori* infection and family history of GC existed five to eight times of getting GC, higher than a control group [17, 20], and these two factors could be interconnected [17]. First of all, the genetic aspect of GC genesis has been explained by the susceptibility of genetic polymorphism of inflammation-related genes, such as *interleukin-1β* gene (*IL-1β*), *tumor necrosis factor-α* gene (*TNF-α*), *IL-6*, *IL-8*, *IL-10*, *Toll-like receptor 4* (*TLR-4*), and transforming growth factor (*TGF*)-*β* [31–33]. However, this gene-related explanation has a problem of reproducibility because test results can be different based on the patient's ethnicity. For instance, genetic polymorphism of *IL-1β* and *TNF-α* was consistently related to GC among Western patients [34, 35], while this phenomenon was inconsistent among Eastern patients [36, 37]. In contrast, *IL-8* and *TGF-β* genetic polymorphism and GC genesis were more consistently related among Eastern patients than Western patients [38–41]. Thus, ethnicity must be considered. These genetic factors were influential when *H. pylori* infection triggered premalignant lesions such as intestinal metaplasia (IM), by inflammation of gastric mucosa. Based on research about risk factors for atrophic gastritis (AG) and IM in subjects without significant gastroduodenal diseases, AG was related to not only *H. pylori* infection but also *H. pylori* toxic factors such as *vacA* m1 and *cagA*. In contrast, risk factors for IM were *H. pylori* infection, smoking, age over 61, spicy food, and *IL-10*-592 C/A [42]. These results suggest that genetic polymorphism is powerful for IM, which increased GC 10.9 times higher than in the absence of IM [42].

To find out the increasing mechanism of GC by family history of GC, a comprehensive approach is necessary on the genetic factors, the environmental factors, and *H. pylori* infection and especially on the interaction between *H. pylori* virulence factors and genetic susceptibility. According to a recent comprehensive study regarding 123 GC patients with GC history and 639 age- and sex-matched control GC patients without family GC history, the rate of IM in the corpus among the GCRs less than 50 years old was higher than that of the control group ($p = 0.018$) [41]. Next, intestinal-type GC patients with a family history of GC spent their youth in rural areas (OR 1.98; 95% CI, 1.02–3.85), and only a few patients showed *TGFB1*–509T (OR 0.47; 95% CI, 0.25–0.86), suggesting that both environmental factors and the genetic polymorphism affect the genesis of intestinal-type GC among GCRs [41].

28.3.2 The Preventive Effect of *H. pylori* Eradication in Subjects with a Family History of Gastric Cancer

There was a single-center, double-blind, placebo-controlled trial from South Korea regarding the preventive effect of *H. pylori* eradication in subjects with a family history of GC [43]. They screened 3100 first-degree relatives of patients with GC and randomly assigned 1838 participants with *H. pylori* infection to receive either eradication therapy (lansoprazole [30 mg], amoxicillin [1000 mg], and clarithromycin [500 mg], each taken twice daily for 7 days) or placebo [43]. The primary outcome was the development of GC. A total of 1676 participants were included in the modified intention-to-treat population for the analysis of the primary outcome (832 in the treatment group and 844 in the placebo group) [43]. During a median follow-up of 9.2 years, GC developed in 10 participants (1.2%) in the treatment group and in 23 (2.7%) in the placebo group (hazard ratio [HR] 0.45; 95% CI, 0.21–0.94; $p = 0.03$ by log-rank test) [43] (Fig. 28.1a). Among the 10 participants in the treatment group in whom GC developed, 5 (50.0%) had persistent *H. pylori* infection. GC developed in 0.8% of participants (5 of 608) in whom *H. pylori* infection was eradicated and in 2.9% of participants (28 of 979) who had a persistent infection (HR 0.27; 95% CI, 0.10–0.70) [43] (Fig. 28.1b) [43]. As *H. pylori* eradication reduced the risk of GC, *H. pylori* eradication treatment is strongly recommended in first-degree relatives of GC with *H. pylori* infection [44].



No. at Risk

Placebo	844	842	804	769	731	701	640	600	515	423	271	194	94	33	1	0
H. pylori treatment	832	832	793	766	727	697	634	593	496	419	275	180	89	31	1	0

No. at Risk

Persistent infection	979	979	937	899	858	819	743	695	588	482	313	214	105	35	0	0
Eradicated infection	608	608	583	565	547	531	494	465	395	340	221	153	76	27	2	0

Fig. 28.1 Cumulative incidence of gastric cancer (GC) according to *H. pylori* eradication status. (**a**) Kaplan–Meier curves for the primary outcome of the development of GC. During a median follow-up of 9.2 years, GC developed in 10 of 832 participants (1.2%) in the treatment group and in 23 of 844 (2.7%) in the placebo group. (**b**) Kaplan–Meier curves for GC according to *H. pylori* eradication status after receipt of *H. pylori* treatment or placebo (a secondary outcome). A total of 1587 participants were included in the analysis after the exclusion of 89 participants who did not have data from a follow-up *H. pylori* test. GC developed in 5 of 608 participants (0.8%) in whom infection was eradicated and in 28 of 979 participants (2.9%) with persistent infection. The inset shows the same data on an enlarged *y*-axis. (Adapted from Choi et al. [43])

28.4 Risk Factors for Gastric Cancer According to the Number of Affected Relatives and According to the Affected Family Member

Even though it is believed that the GC risk increases with an increasing number of affected relatives or it could be different depending on the affected family member, there have been few reports regarding this factor [8, 18]. For instance, the risk associated with GC occurrence in any first-degree relatives tended to be greater for women than for men [8, 18]. In addition, the GC risk was higher for subjects having an affected mother than an affected father (OR 3.78; 95% CI, 1.49–9.59 vs. 2.85, 1.46–5.56) [18]. Overall, a parental history of GC increased the GC risk (OR 3.30; 95% CI, 1.89–5.75) [18]. The GC risk for subjects with a sibling with GC also increased significantly (OR 2.44; 95% CI, 1.25–4.74), but the risk was not as strong as that for subjects with an affected parent [18]. In addition, the GC risk increased for those that had one first-degree family member affected with GC (OR 2.72; 95% CI, 1.72–4.32), but the risk increased further for subjects with two or more first-degree relatives with GC (OR 9.60; 95% CI, 1.18–73.35) [18]. Recently, our group further performed a comprehensive analysis in a total of 1058 GC patients and 1268 controls according to the presence or absence of a first-degree relative diagnosed with GC (GC-relative) [45]. Since the GC risk increases with an increasing number of affected relatives, the family history-positive group was divided into two categories, having one GC-relative and having two or more GC-relatives, to evaluate the familial aggregation of GC [45] (Table 28.1). Residence in rural areas in child-

Table 28.1 Comparison of clinicopathologic variables with regard to the number of affected relatives of gastric cancer

	Group with 1 GC-relative			Group with ≥2 GC-relatives			
Variables [reference]	Control (*n* = 308)	GC (*n* = 188)	OR (95% CI)	Control (*n* = 47)	GC (*n* = 30)	OR (95% CI)	$p_{\text{Interaction}}$
Age, y, median (IQR)	51 (43–60)	62 (52–69)	**1.07 (1.05–1.09)**	57 (49–65)	63 (55.0–72.3)	**1.08 (1.02–1.13)**	0.807
Male gender [women]	134 (43.5)	122 (64.9)	**2.38 (1.64–3.46)**	14 (30.4)	22 (73.3)	**5.87 (2.13–16.19)**	0.102
Rural residency [urban]	141 (48.1)	120 (66.7)	**2.14 (1.46–3.15)**	22 (52.4)	26 (89.7)	**7.54 (1.98–28.69)**	0.076
Smoker (current/ex) [never]	108 (35.6)	105 (55.9)	**2.23 (1.54–3.23)**	14 (30.4)	22 (73.3)	**6.48 (2.33–18.02)**	0.054
Alcohol [never]	116 (37.7)	95 (50.8)	**1.71 (1.18–2.47)**	12 (25.5)	23 (76.7)	**9.58 (3.29–27.95)**	**0.026**
Income <$5000/month [≥$5000/month]	189 (68.2)	144 (83.7)	**2.42 (1.50–3.90)**	25 (71.4)	22 (78.6)	1.41 (0.44–4.50)	0.584
Spicy food [no]	208 (72.0)	138 (77.1)	1.31 (0.85–2.02)	32 (78.0)	28 (96.6)	7.64 (0.91–64.03)	0.111
Fruit intake ≥3/week [<3/week]	41 (14.0)	44 (24.7)	**1.50 (1.15–1.96)**	13 (31.7)	8 (27.6)	1.26 (0.68–2.33)	0.155
Non-B blood [B]	210 (73.2)	143 (76.9)	1.20 (0.78–1.84)	34 (79.1)	21 (75.0)	0.77 (0.25–2.38)	0.475
Antral IM [none]	91 (29.9)	127 (67.9)	**5.18 (3.49–7.70)**	17(38.6)	18 (62.1)	2.46 (0.94–6.40)	0.158
Corpus IM [none]	67 (22.0)	78 (41.7)	**2.62 (1.76–3.90)**	9 (20.0)	15 (50.0)	**3.60 (1.32–9.80)**	0.562
Current/past HP infection	221 (71.8)	170 (90.4)	**3.70 (2.14–6.38)**	34 (72.3)	22 (73.3)	1.05 (0.37–2.95)	**0.035**

Table 28.1 (continued)

Variables [reference]	Group with 1 GC-relative			Group with ≥2 GC-relatives			$p_{\text{Interaction}}$
	Control (n = 308)	GC (n = 188)	OR (95% CI)	Control (n = 47)	GC (n = 30)	OR (95% CI)	
cag A [negative]	71 (29.0)	76 (44.4)	**1.94 (1.28–2.91)**	9 (26.5)	14 (46.7)	2.43 (0.85–6.92)	0.691
TGFB1–509 C/C [reference]	78 (25.7)	57 (31.1)	1 (reference)	16 (35.6)	8 (26.7)	1 (reference)	
TGFB1–509 C/T	150 (49.5)	82 (44.8)	0.74 (0.48–1.14)	22 (48.9)	15 (50.0)	1.36 (0.47–3.99)	0.330
TGFB1–509 T/T	75 (24.8)	44 (24.0)	0.80 (0.48–1.33)	7 (15.6)	7 (23.3)	2.00 (0.50–7.70)	0.214
*IL-1RN*2* carriers [non-carrier]	42 (13.9)	19 (10.2)	0.71 (0.40–1.27)	5 (10.6)	4 (13.3)	1.29 (0.32–5.26)	0.440
IL-1B-511 T allele [C/C]	247 (81.3)	145 (77.9)	0.81 (0.52–1.27)	40 (85.1)	24 (80.0)	0.70 (0.21–2.33)	–
Affected family member							
Mother [no]	98 (31.8)	46 (24.6)	0.70 (0.46–1.05)	22 (46.8)	13 (43.3)	0.77 (0.46–2.89)	0.333
Father [no]	128 (41.6)	72 (38.5)	0.89 (0.62–1.29)	20 (42.6)	11 (36.7)	0.78 (0.31–2.00)	0.797
Sister [no]	36 (11.7)	21 (11.2)	0.96 (0.54–1.69)	16 (34.0)	12 (40.0)	1.29 (0.50–3.33)	0.594
Brother [no]	45 (14.6)	41 (21.9)	**1.64 (1.03–2.62)**	30 (63.8)	19 (63.3)	0.98 (0.38–2.54)	0.340
Offspring [no]	1 (0.3)	7 (3.7)	11.94 (1.46–97.82)	0	0	–	–
Intestinal:diffuse	0	112:70	–	0	19:11	–	0.851
Non-cardia:cardia	0	167:20	–	0	26:4	–	0.753

Adapted from Choi et al. [45]
Data are presented as *n* (%) unless otherwise indicated
The bold style indicates the statistical significance
GC gastric cancer, *GC-relative* first-degree relative diagnosed with gastric cancer, *HP Helicobacter pylori*, *AG* atrophic gastritis, *IM* intestinal metaplasia, *IL* interleukin, *mo* month, *SD* standard deviation, *TGF* transforming growth factor, *OR* odds ratio, *CI* confidence interval

hood and current or ex-smoking were more likely to increase the risk of GC in the ≥2 GC-relative group (OR 2.14 vs. 7.54; *p* for interaction = 0.076 and OR 2.23 vs. 6.48; *p* for interaction = 0.054) [45] (Table 28.1). Moreover, there was an interaction between alcohol consumption and GC risk in the ≥2 GC-relative group. That is, OR for drinking in this group was sixfold as high as that of one GC-relative group (1.71 vs. 9.58, *p* for interaction = 0.026). When the amount of alcohol was stratified, heavy drinkers (≥144 g of ethanol/week) showed the highest synergistic effect compared to none (24 g of ethanol/week <), light (144 g of ethanol/week <), and ex-alcohol user. Contrary to this, *H. pylori* infection was more closely associated with GC patients in the single GCR group than in the at least two GCR groups [45] (Table 28.2). When multivariable analyses were performed in each group with one GCR and two or more GCRs, respectively, the significant risk factors for GC in subjects with single GCR were almost the same as the analysis of the total family history-positive subjects [45] (Table 28.2). Only alcohol consumption was excluded from the risk factors. However, when the subjects were restricted to those with two or more GCRs, having *TGFB1*–509T/T was newly incorporated into the risk factors for GC along with rural residency in childhood, alcohol consumption, moderate-to-strong spicy food ingestion, and *cagA* positivity [45] (Table 28.3). When the characteristics of GC patients were evaluated accord-

Table 28.2 ORs for gastric cancer with regard to the amount of alcohol consumption and number of affected relatives of gastric cancer

	Group with 1 GC-relative			Group with ≥2 GC-relatives			
Alcohol^a	Control (*n* = 308)	GC (*n* = 188)	OR (95% CI)	Control (*n* = 47)	GC (*n* = 30)	OR (95% CI)	$p_{\text{interaction}}$
Never/ rare	192 (62.3)	92 (49.2)	1 (reference)	35 (74.5)	7 (23.3)	1 (reference)	
Light	60 (19.5)	35 (18.7)	1.22 (0.75–1.98)	10 (21.3)	9 (30.0)	**4.50 (1.34–15.12)**	0.392
Heavy	31 (10.1)	29 (15.5)	**1.95 (1.11–3.43)**	1 (2.1)	11 (36.7)	**55.0 (6.08–497.42)**	**0.022**
Ex	25 (8.1)	31 (16.6)	**2.59 (1.45–4.63)**	1 (2.1)	3 (10.0)	**15.0 (1.36–166.05)**	0.456

Adapted from Choi et al. [45]

The bold style indicates the statistical significance

GC gastric cancer, *GC-relative* first-degree relative diagnosed with gastric cancer, *OR* odds ratio, *CI* confidence interval

[a] Never/rare drinker refers to a non-drinker or those who drink ≤0.5 U/week; 0.5 U/week < light drinker <12 U/week; heavy drinker ≥12 U/week (1 U = 12 g of ethanol)

Table 28.3 Risk factors for family history-positive gastric cancer according to the number of affected first-degree relatives by multivariable analyses

	GC-relative = 1^a		Variables	GC-relative ≥2^c	
Variables	*p*-value	aOR^b (95% CI)		*p*-value	aOR^b (95% CI)
Male gender	**0.002**	2.24 (1.33–3.77)			
Age	**<0.001**	1.06 (1.034–1.09)			
Antral IM	**<0.001**	2.80 (1.65–4.75)			
Current income (<$5000/ month)	**0.017**	2.19 (1.15–4.16)			
HP infection	**0.024**	2.50 (1.13–5.55)			
cagA	**0.006**	2.17 (1.25–3.77)	*cagA*	**0.038**	9.06 (1.12–72.97)
Rural residency in childhood	**0.002**	1.85 (1.10–3.12)	Rural residency in childhood	**0.004**	26.49 (2.90–241.99)
			Alcohol consumption	**0.002**	27.83 (3.37–230.16)
			≥Moderate spicy food ingestion	**0.004**	65.74 (3.91–105.99)
			TGFB1–509 C/T	0.267	3.42 (0.39–30.05)
			*TGFB1–509*T/T	**0.029**	23.74 (1.37–410.91)

Adapted from Choi et al. [45]

GC-relative first-degree relative diagnosed with gastric cancer, *aOR* adjusted odds ratio, *CI* confidence interval, *HP Helicobacter pylori*, *IM* intestinal metaplasia, *mo* month, *TGF* transforming growth factor

[a] For 308 control subjects and 188 patients with gastric cancer

[b] Logistic model including terms for age, gender, *H. pylori* infection, *H. pylori* infection by gender, current income, residency during childhood, smoker, alcohol consumption, alcohol consumption by smoker, diet of salty/spicy food, fruit intake, intestinal metaplasia, non-B blood type, *cagA*, *IL-1RN* *2 carriers, *TGFB1*-C509T polymorphism (reference *TGFB1*–509C/C)

[c] For 47 control subjects and 30 patients with gastric cancer

Table 28.4 Characteristics of patients with gastric cancer according to different parental history of gastric cancer

	Affected family member				
Variables	Mother ($n = 59$)	Father ($n = 79$)	Only sibling/offspring ($n = 75$)	p-value[a]	p-value[b]
Male gender, *n* (%)	41 (69.5)	53 (67.1)	47 (62.7)	0.694	0.764
Age at diagnosis, mean (SD)	62.2 (9.2)	57.0 (11.4)	64.5 (9.8)	**<0.001**	**0.012**
GC-relative ≥2, *n* (%)	13 (22.0)	7 (8.9)	6 (8.0)	**0.025**	**0.030**
No. of affected family					
Member, mean (SE)	1.36 (0.98)	1.10 (0.34)	1.08 (0.27)	**0.012**	**0.034**
HP infection, *n* (%)	64 (85.3)	72 (91.1)	52 (88.1)	0.694	0.563
cagA, *n* (%)	17 (30.9)	33 (46.5)	37 (52.9)	**0.045**	0.076
Rural residency, *n* (%)	39 (68.4)	48 (65.8)	53 (71.6)	0.745	0.748
Alcohol consumption, *n* (%)	29 (49.2)	39 (49.4)	30 (40.5)	0.477	0.980
Spicy food ingestion, *n* (%)	44 (77.2)	60 (83.3)	58 (77.3)	0.592	0.381
Intestinal type, *n* (%)	37 (63.8)	48 (62.3)	46 (63.0)	0.985	0.862
Cardiac cancer, *n* (%)	5 (8.5)	5 (6.3)	14 (18.7)	**0.039**	0.631
*TGFB1–509*T allele, *n* (%)	43 (74.1)	55 (71.4)	46 (63.0)	0.340	0.727
*IN-1RN *2* carrier, *n* (%)	7 (12.1)	5 (6.3)	10 (13.5)	0.310	0.240
*IL-1B 511*T allele, *n* (%)	45 (77.6)	60 (75.9)	60 (81.1)	0.738	0.823

Adapted from Choi et al. [45]

The bold style indicates the statistical significance

HP Helicobacter pylori, *IL* interleukin, *SD* standard deviation, *SE* standard error, *TGF* transforming growth factor

[a] Compared among the three groups (maternal, paternal, and sibling/offspring)

[b] Compared between maternal and paternal

ing to affected relatives, the GC group with a maternal history had an overall larger number of affected relatives than the group with an affected father or the group with affected siblings or offspring (1.36 ± 0.98 vs. 1.10 ± 0.34 and 1.08 ± 0.27, $p = 0.012$ and $p = 0.034$, respectively) [45] (Table 28.4). When multivariable logistic analysis adjusted for age, gender, *H. pylori* infection, and alcohol consumption was performed in GC patients with a family history, positive maternal history was a risk factor for having two or more GCRs (maternal history, OR 4.30; 95% CI, 1.34–13.79, $p = 0.014$; *H. pylori* infection, OR 0.24; 95% CI, 0.08–0.82, $p = 0.011$; alcohol consumption, OR 1.89; 95% CI, 1.14–7.33, $p = 0.025$) [45] (Table 28.4). Moreover, the GC group with an affected father was younger than the group with an affected mother and the group with affected only siblings (57.0 ± 1.3 vs. 62.2 ± 1.2 and 64.5 ± 1.1, $p = 0.012$ and $p < 0.001$, respectively) [45] (Table 28.4). These results suggest that subjects with a family history may be a heterogeneous group in terms of GC susceptibility. In particular, subjects with ≥2 GCRs may need a risk stratification including *TGFB1*–509T/T or amount of alcohol consumption [45]. Taken together, the number of affected GC family and affected relatives might be an interesting topic, which could uncover the mechanism behind the GCRs, and it needs more research in the future.

28.5 Family-Based Exome Sequencing Combined with Linkage Analyses Identifies Rare Susceptibility Variants of *MUC4* for Gastric Cancer

As the family history of GC increased, the incidence of GC development our team tried to identify GC-associated germline variants with a high effect size [46] by linkage and association analyses based on whole-exome sequencing (WES) of subjects with familial clustering of GC [47]. WES of DNAs from the blood of 19 GC patients and 36 unaffected family members from 14 families with two or more GC patients were tested [47]. Linkage analysis combined with association tests were performed using Pedigree Variant Annotation, Analysis, and Search Tool (pVAAST) software. Based on the logarithm of odds (LOD) and permutation-based composite likelihood ratio test (CLRT) from pVAAST, *MUC4* was identified as a predisposing gene (LOD p-value = 1.9×10^{-5}; permutation-based p-value of CLRT $\leq 9.9 \times 10^{-9}$). In a larger cohort consisting of 597 GC patients and 9759 healthy controls genotyped with SNP array, our team discovered common variants in *MUC4* regions (rs148735556, rs11717039, and rs547775645) significantly associated with GC supporting the association of *MUC4* with GC [47]. The *MUC4* variants were found in higher frequency in The Cancer Genome Atlas Study (TCGA) germline samples of patients with multiple cancer types. Immunohistochemistry indicated that *MUC4* was downregulated in the noncancerous gastric mucosa of subjects with *MUC4* germline missense variants, suggesting that loss of the protective function of *MUC4* predisposes an individual to GC [47]. Rare variants in *MUC4* can be novel GC susceptibility loci in Koreans possessing the familial clustering of GC [47]. MUC4 is a large, heavily glycosylated transmembrane mucin, varying from 550 to 930 kDa, due to the polymorphic variable number tandem repeat region [48]. MUC4 is expressed in the normal epithelium of several organs, including the stomach, intestine, and mammary glands, and protects and lubricates the epithelium [48]. *MUC4* shares structural similarities with *MUC1* but also possesses unique regions, including three EGF-like domains, which are required for the activation of ErbB2. Maybe it is valuable to investigate its role in the detection of possible development of GC in subjects with two or more first-degree relatives of GC.

28.6 Family History of Gastric Cancer as a Risk Factor for Intestinal Metaplasia

There is a high possibility of GC development if a patient has a family history of GC, so it has been predicted that gastric mucosal inflammation due to *H. pylori* infection would be more severe than a patient without a family history. According to a meta-analysis result of six research studies that were published until 2009, the rates of AG and IM in the presence of the family history of GC were 2.2 times and 1.98 times higher than in the absence of the family history of GC, respectively [12]. However, research from Iran in 2012 reported that a family history of GC only increased IM, but not AG [49]. In addition, Oh et al. suggested that first-degree relatives had adjusted OR of 2.69 (95% CI, 1.06–6.80, $p = 0.037$) for antral IM in the male population in multivariate regression analysis, but there was no increase in AG [50]. Meanwhile, based on analyses of endoscopic observations of 4023 patients who received health screening in Korea, 11% of entire patients had a family history of GC, and the endoscopic positive rates of AG or IM were 40.7% and 12.5%, respectively [29]. In this research, risk factors of AG were the age from 40 to 59 (OR 2.55), the age over 60 years old (OR 5.00), male (OR 1.38), serologically positive *H. pylori* (OR 1.41), and an academic background less than a college education (OR 1.35) [29]. Interestingly, the risk factors of IM were very similar to those of AG, but dairy product consumption (OR 1.40) and family history of GC (OR 1.48) were additionally found [29]. Taken together, it indicates that somehow IM can frequently occur among people with a family history of GC [12, 29, 50], which can increase the risk of GC in the GCRs. As the most important risk factor for IM is *H. pylori* infection [42] and family history of GC is also an important risk factor for GC, the importance of *H. pylori* eradication in the GCRs before the development of IM is suggested.

28.7 Prognosis of Gastric Cancer in the Relatives of Gastric Cancer

The prognosis of a GC patient who has a family history of GC tends to be better than a patient without family history [51–53]. According to a report from Korea, there were many cases of shallow GC infiltration in GC cases of GCRs [51]. In addition, the National Cancer Center in Korea reported that 20.6% (263 patients) of 1273 GC patients had a family history of GC and 71.8% of these GCRs had intestinal-type GC, while only 55.1% of patients without the history had intestinal type (p = 0.015) [52]. Also, the patients with a family history showed better prognosis, so their disease-free survival rate, recurrence-free survival rate, and overall survival rate were better, and they showed meaningful prognostic differences, especially in the stages of GC III and IV [52]. Moreover, the Italian group reported that 21.9% of GC patients had a family history of GC, and 71.8% of the patients with a family history showed intestinal-type GC, while only 55.1% of patients without the history had the same type [53, 54]. However, an opposite result was reported from Japan that the prognosis of GC patients with a family history was worse than patients without the history [55]. Also, a report from Taiwan showed that the prognosis was not affected by the family history of GC [56]. Our team also researched this issue with a hypothesis that a family history of GC would affect the TNM stage of GC, and this influence might be associated with the CC genotype of *TGFB1*–509 polymorphism [57]. When 1143 GC patients were classified depending on family history of GC, the proportion of stage I–II GC was significantly higher in 216 patients (18.9%) with a family history of GC than in those without a family history (83.8% vs. 74.9%, $p = 0.005$) [57]. However, except for the tumor stage, there were no significant differences in the characteristics between patients with and without a family history of GC [57]. Disappointingly, the association between a family history and stages I–II of GC was not significant according to *TGFB1* C-509T polymorphism and sex. Finally, a family history did not affect the overall survival rate of GC in all stages and each stage. Furthermore, the overall survival rates were not significantly different between the CC and CT/TT genotypes of the *TGFB1*–509 polymorphism [57].

These various results might be caused by the differences in research methods or applicants or the presence of confounding factors. Meanwhile, a recent meta-analysis of five reports showed that family history is a beneficial factor for GC [58]. Perhaps, subjects do frequent health screenings due to their family history of GC and show good health-related behaviors, such as non-smoking, non-alcoholic drinking, and consistent exercise, and the patients with a family history of GC often show microsatellite instability (MSI) and mismatch repair proteins that are related to the good prognosis [52]. This prediction requires further research, but it is a positive phenomenon that GC patients with a family history show a good GC prognosis.

28.8 Guideline of Gastric Cancer Screening in Case of Family History of Gastric Cancer

Normally, diet therapy is important to prevent GC. Taking fresh food, minimizing salty and spicy food consumption, and avoiding stimulant food are recommended [59]. Next, *H. pylori* eradication is the most important recommendation among ages in 20s and 30s because it can prevent future IM via early eradication [21, 41]. However, when a survey was conducted among gastroenterology experts in Korea about the statement “*H. pylori* eradication therapy can be helpful to prevent GC among *H. pylori*-infected patients with immediate family history on GC,” responses were “totally agree” (17.2%), “generally agree” (58.6%), “partially agree” (17.2%), “generally disagree” (3.5%), “totally disagree” (3.5%), and “do not know” (0%). Thus, it can be inferred that eradication therapy is not much recommended in the clinical field [60]. However, this attitude changed mainly due to the evidence that *H. pylori* eradication reduced the risk of GC [44]. In 2020 Korean guideline, *H. pylori* eradication treatment is strongly recommended in first-degree relatives of GC with *H. pylori* infection [44].

Once IM occurs, periodical gastroscopy is necessary to find an early lesion that is treatable with endoscopy. Nonetheless, the number of gastroscopies can be varied among countries due to a cost–benefit matter. For instance, a cohort study in the United States reported that the possibilities of GC progression from AG and IM are 0.3% and 1.0%, respectively [61]. Also, a cohort study from the Netherlands reported that the rates of GC development from AG and IM within 10 years were 0.8% and 1.8%, respectively. Because of these differences, establishing a global consensus is difficult. However, the recent European guideline has selected OLGA, OLGIM, serum pepsinogen level, serological *H. pylori* test, and family history on GC, which are helpful in setting up a high-risk group for GC [62]. This guideline recommends having a gastroscopy every 3 years, if a patient has broad AG or IM. However, nations with high GC occurrence, such as Korea, China, and Japan, emphasize patients to have periodic gastroscopy, since it is cost-effective [63–67]. According to a Korean report in 2012, early gastric cancer (EGC) rates of a group that got gastroscopy annually and a group that had gastroscopy every 2 years were 98.6% and 80.7%, respectively, and the rates of treatable cancer with gastroscopy were 56.9% and 33.3%, respectively [68]. In comparison, the EGC rate of a group whose gastroscopy interval was longer than 2 years was 54.6% [68]. In addition, the 5-year survival rates of groups that received gastroscopy annually, every 2 years, and longer intervals than 2 years were 98.5%, 92%, and 86.1%, respectively [68]. Therefore, it is effective to receive gastroscopy annually in case of high-risk group of GC, because endoscopically treatable EGC can be detected [16].

28.9 Conclusions

Family history is important as a risk factor for GC. This factor is partially explained by genetic factors and environmental factors, such as dietary habit, and the factor is also related to familial clustering of *H. pylori* infection. First-degree family history of GC itself increases GC genesis by two to three times, and this genesis increases by 5.88 times if *H. pylori* infection exists, too. Thus, *H. pylori* eradication is the most important priority for first-degree family members, who are less than 40s, especially those in their 20s and 30s, of GC patients. This early *H. pylori* eradication can avoid future IM and restricts the synergistic effect of *H. pylori* infection and immediate family history of GC. Thus, most guidelines strongly recommend *H. pylori* eradication in the first-degree relatives of GC with *H. pylori* infection. Even after *H. pylori* is eradicated, the cascade can proceed, especially, when IM has developed already. Thus, it is important to receive gastroscopy periodically to find out early lesion that can be treated with endoscopy [16].

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209–49.
2. Statistics Korea. Causes of death statistics in 2020. http://kostat.go.kr/portal/eng/pressReleases/1/index.board?bmode=read&bSeq=&aSeq=414516&pageNo=5&rowNum=10&navCount=10&currPg=&searchInfo=&sTarget=title&sTxt=deat. Accessed 15 Nov 2022.
3. Charvat H, Sasazuki S, Inoue M, Iwasaki M, Sawada N, Shimazu T, et al. Prediction of the 10-year probability of gastric cancer occurrence in the Japanese population: the JPHC study cohort II. Int J Cancer. 2016;138:320–31.
4. Inoue M, Tsugane S. Epidemiology of gastric cancer in Japan. Postgrad Med J. 2005;81:419–24.
5. Kang MJ, Jung KW, Bang SH, Choi SH, Park EH, Yun EH, et al. Cancer Statistics in Korea: Incidence, Mortality, Survival, and Prevalence in 2020. Cancer Res Treat. 2023;55:385–99.
6. Joossens JV, Hill MJ, Elliott P, Stamler R, Lesaffre E, Dyer A, et al. Dietary salt, nitrate and stomach cancer mortality in 24 countries. European Cancer Prevention (ECP) and the INTERSALT Cooperative Research Group. Int J Epidemiol. 1996;25:494–504.
7. Ladeiras-Lopes R, Pereira AK, Nogueira A, Pinheiro-Torres T, Pinto I, Santos-Pereira R, et al. Smoking and gastric cancer: systematic review and meta-analysis of cohort studies. Cancer Causes Control. 2008;19:689–701.
8. Palli D, Galli M, Caporaso NE, Cipriani F, Decarli A, Saieva C, et al. Family history and risk of stom-

ach cancer in Italy. Cancer Epidemiol Biomark Prev. 1994;3:15–8.

9. Foschi R, Lucenteforte E, Bosetti C, Bertuccio P, Tavani A, La Vecchia C, et al. Family history of cancer and stomach cancer risk. Int J Cancer. 2008;123:1429–32.
10. Lee HJ, Yang HK, Ahn YO. Gastric cancer in Korea. Gastric Cancer. 2002;5:177–82.
11. Zanghieri G, Di Gregorio C, Sacchetti C, Fante R, Sassatelli R, Cannizzo G, et al. Familial occurrence of gastric cancer in the 2-year experience of a population-based registry. Cancer. 1990;66:2047–51.
12. Rokkas T, Sechopoulos P, Pistiolas D, Margantinis G, Koukoulis G. *Helicobacter pylori* infection and gastric histology in first-degree relatives of gastric cancer patients: a meta-analysis. Eur J Gastroenterol Hepatol. 2010;22:1128–33.
13. Chang YW, Han YS, Lee DK, Kim HJ, Lim HS, Moon JS, et al. Role of *Helicobacter pylori* infection among offspring or siblings of gastric cancer patients. Int J Cancer. 2002;101:469–74.
14. Niv Y. Family history of gastric cancer: should we test and treat for *Helicobacter pylori*. J Clin Gastroenterol. 2003;36:204–8.
15. Fujimoto Y, Furusyo N, Toyoda K, Takeoka H, Sawayama Y, Hayashi J. Intrafamilial transmission of *Helicobacter pylori* among the population of endemic areas in Japan. Helicobacter. 2007;12:170–6.
16. Yoon H, Kim N. Diagnosis and management of high risk groups of gastric cancer. Gut Liver. 2015;9:5–17.
17. Brenner H, Arndt V, Stürmer T, Stegmaier C, Ziegler H, Dhom G. Individual and joint contribution of family history and *Helicobacter pylori* infection to the risk of gastric carcinoma. Cancer. 2000;88:274–9.
18. Shin CM, Kim N, Yang HJ, Cho SI, Lee HS, Kim JS, et al. Stomach cancer risk in gastric cancer relatives: interaction between *Helicobacter pylori* infection and family history of gastric cancer for the risk of stomach cancer. J Clin Gastroenterol. 2010;44:e34–9.
19. Brenner H, Bode G, Boeing H. *Helicobacter pylori* infection among offspring of patients with stomach cancer. Gastroenterology. 2000;118:31–5.
20. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of *Helicobacter pylori* infection—the Maastricht IV/ Florence Consensus Report. Gut. 2012;61:646–64.
21. Fock KM, Talley N, Moayyedi P, Hunt R, Azuma T, Sugano K, et al. Asia-Pacific consensus guidelines on gastric cancer prevention. J Gastroenterol Hepatol. 2008;23:351–65.
22. Kim N, Kim JJ, Choe YH, Kim HS, Kim JI, Chung IS, et al. Diagnostic and treatment guideline of *Helicobacter pylori* infection in Korea. Korean J Gastroenterol. 2009;54:269–78.
23. Yim JY, Kim N, Choi SH, Kim YS, Cho KR, Kim SS, et al. Seroprevalence of *Helicobacter pylori* in South Korea. Helicobacter. 2007;12:333–40.
24. Lim SH, Kwon JW, Kim N, Kim GH, Kang JM, Park MJ, et al. Prevalence and risk factors of *Helicobacter pylori* infection in Korea: nationwide multicenter study over 13 years. BMC Gastroenterol. 2013;13:104.
25. Kim JH, Kim HY, Kim NY, Kim SW, Kim JG, Kim JJ, et al. Seroepidemiological study of *Helicobacter pylori* infection in asymptomatic people in South Korea. J Gastroenterol Hepatol. 2001;16:969–75.
26. Bures J, Kopácová M, Koupil I, Vorísek V, Rejchrt S, Beránek M, et al. Epidemiology of *Helicobacter pylori* infection in The Czech Republic. Helicobacter. 2006;11:56–65.
27. Bureš J, Kopáčová M, Koupil I, Seifert B, Skodová Fendrichová M, Spirková J, et al. Significant decrease in prevalence of *Helicobacter pylori* in The Czech Republic. World J Gastroenterol. 2012;18:4412–8.
28. Leung WK, Ng EK, Lam CC, Chan KF, Chan WY, Auyeung AC, et al. *Helicobacter pylori* infection in 1st degree relatives of Chinese gastric cancer patients. Scand J Gastroenterol. 2006;41:274–9.
29. Joo YE, Park HK, Myung DS, Baik GH, Shin JE, Seo GS, et al. Prevalence and risk factors of atrophic gastritis and intestinal metaplasia: a nationwide multicenter prospective study in Korea. Gut Liver. 2013;7:303–10.
30. Marcos-Pinto R, Carneiro F, Dinis-Ribeiro M, Wen X, Lopes C, Figueiredo C, et al. First-degree relatives of patients with early-onset gastric carcinoma show even at young ages a high prevalence of advanced OLGA/ OLGIM stages and dysplasia. Aliment Pharmacol Ther. 2012;35:1451–9.
31. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature. 2000;404:398–402.
32. El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, et al. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. Gastroenterology. 2003;124:1193–201.
33. Canedo P, Figueiredo C, Machado JC. After *Helicobacter pylori*, genetic susceptibility to gastric carcinoma revisited. Helicobacter. 2007;12 (Suppl 2):45–9.
34. Kamangar F, Cheng C, Abnet CC, Rabkin CS. Interleukin-1B polymorphisms and gastric cancer risk—a meta-analysis. Cancer Epidemiol Biomark Prev. 2006;15:1920–8.
35. Gorouhi F, Islami F, Bahrami H, Kamangar F. Tumour-necrosis factor-a polymorphisms and gastric cancer risk: a meta-analysis. Br J Cancer. 2008;98:1443–51.
36. Lee SG, Kim B, Choi W, Lee I, Choi J, Song K. Lack of association between pro-inflammatory genotypes of interleukin-1 (*IL-1B-31* C/+ and *IL-1RN* *2/*2) and gastric cancer/duodenal ulcer in Korean population. Cytokine. 2003;21:167–71.

37. Kim N, Cho SI, Yim JY, Kim JM, Lee DH, Park JH, et al. The effects of genetic polymorphisms of *IL-1* and *TNF-A* on *Helicobacter pylori*-induced gastroduodenal diseases in Korea. Helicobacter. 2006;11:105–12.
38. Savage SA, Abnet CC, Mark SD, Qiao YL, Dong ZW, Dawsey SM, et al. Variants of the IL8 and IL8RB genes and risk for gastric cardia adenocarcinoma and esophageal squamous cell carcinoma. Cancer Epidemiol Biomark Prev. 2004;13:2251–7.
39. Song JH, Kim SG, Jung SA, Lee MK, Jung HC, Song IS. The interleukin-8-251 AA genotype is associated with angiogenesis in gastric carcinogenesis in *Helicobacter pylori*-infected Koreans. Cytokine. 2010;51:158–65.
40. Jin G, Wang L, Chen W, Hu Z, Zhou Y, Tan Y, et al. Variant alleles of *TGFB1* and *TGFBR2* are associated with a decreased risk of gastric cancer in a Chinese population. Int J Cancer. 2007;120:1330–5.
41. Shin CM, Kim N, Lee HS, Lee DH, Kim JS, Jung HC, et al. Intrafamilial aggregation of gastric cancer: a comprehensive approach including environmental factors, *Helicobacter pylori* virulence and genetic susceptibility. Eur J Gastroenterol Hepatol. 2011;23:411–7.
42. Kim N, Park YS, Cho SI, Lee HS, Choe G, Kim IW, et al. Prevalence and risk factors of atrophic gastritis and intestinal metaplasia in a Korean population without significant gastroduodenal disease. Helicobacter. 2008;13:245–55.
43. Choi IJ, Kim CG, Lee JY, Kim YI, Kook MC, Park BR, et al. Family history of gastric cancer and *Helicobacter pylori* treatment. N Engl J Med. 2020;382:427–36.
44. Jung HK, Kang SJ, Lee YC, Yang HJ, Park SY, Shin CM, et al. Evidence-based guidelines for the treatment of *Helicobacter pylori* infection in Korea 2020. Gut Liver. 2021;15:168–95.
45. Choi YJ, Kim N, Jang W, Seo B, Oh S, Shin CM, et al. Familial clustering of gastric cancer: a retrospective study based on the number of first-degree relatives. Medicine. 2016;95:e3606.
46. Luzon-Toro B, Gui H, Ruiz-Ferrer M, Sze-Man Tang C, Fernandez RM, Sham PC, et al. Exome sequencing reveals a high genetic heterogeneity on familial Hirschsprung disease. Sci Rep. 2015;5:16473.
47. Choi YJ, Ohn JH, Kim N, Kim W, Park K, Won S, et al. Family-based exome sequencing combined with linkage analyses identifies rare susceptibility variants of *MUC4* for gastric cancer. PLoS One. 2020;15:e0236197.
48. Chaturvedi P, Singh AP, Batra SK. Structure, evolution, and biology of the MUC4 mucin. FASEB J. 2008;22:966–81.
49. Mansour-Ghanaei F, Joukar F, Baghaei SM, Yousefi-Mashhoor M, Naghipour MR, Sanaei O, et al. Gastric precancerous lesions in first degree relatives of patients with known gastric cancer: a cross-sectional prospective study in Guilan Province, north of Iran. Asian Pac J Cancer Prev. 2012;13:1779–82.
50. Oh S, Kim N, Yoon H, Choi YJ, Lee JY, Park KJ, et al. Risk factors of atrophic gastritis and intestinal metaplasia in first-degree relatives of gastric cancer patients compared with age-sex matched controls. J Cancer Prev. 2013;18:149–60.
51. Park SH, Yu W, Chung HY. Characteristics of gastric cancer patients with family history. J Korean Surg Soc. 2001;60:309–13.
52. Han MA, Oh MG, Choi IJ, Park SR, Ryu KW, Nam BH, et al. Association of family history with cancer recurrence and survival in patients with gastric cancer. J Clin Oncol. 2012;30:701–8.
53. Palli D, Russo A, Saieva C, Salvini S, Amorosi A, Decarli A. Dietary and familial determinants of 10-year survival among patients with gastric carcinoma. Cancer. 2000;89:1205–13.
54. Bernini M, Barbi S, Roviello S, Scarpa A, Moore P, Pedrazzani C, et al. Family history of gastric cancer: a correlation between epidemiologic findings and clinical data. Gastric Cancer. 2006;9:9–13.
55. Yatsuya H, Toyoshima H, Mizoue T, Kondo T, Tamakoshi K, Hori Y, et al. Family history and the risk of stomach cancer death in Japan: differences by age and gender. Int J Cancer. 2002;97:688–94.
56. Lee WJ, Hong RL, Lai IR, Chen CN, Lee PH, Huang MT. Clinicopathologic characteristics and prognoses of gastric cancer in patients with a positive familial history of cancer. J Clin Gastroenterol. 2003;36:30–3.
57. Kim HJ, Kwon M, Kim N, Lee JB, Won SH. The influence of family history on stage and survival of gastric cancer according to the *TGFB1* C-509T polymorphism in Korea. Gut Liver. 2020;14:79–88.
58. Oh MG, Kim JH, Hann MA, Park J, Ryu SY, Choi SW. Family history and survival of patients with gastric cancer: a meta-analysis. Asian Pac J Cancer Prev. 2014;15:3465–70.
59. Compare D, Rocco A, Nardone G. Risk factors in gastric cancer. Eur Rev Med Pharmacol Sci. 2010;14:302–8.
60. Kim SG, Jung HK, Lee HL, Jang JY, Lee H, Kim CG, et al. Guidelines for the diagnosis and treatment of *Helicobacter pylori* infection in Korea, 2013 revised edition. Korean J Gastroenterol. 2013;62:3–26.
61. Yeh JM, Hur C, Kuntz KM, Ezzati M, Goldie SJ. Cost-effectiveness of treatment and endoscopic surveillance of precancerous lesions to prevent gastric cancer. Cancer. 2010;116:2941–53.
62. Dinis-Ribeiro M, Areia M, de Vries AC, Marcos-Pinto R, Monteiro-Soares M, O'Connor A, et al. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European society of gastrointestinal endoscopy (ESGE), European helicobacter study group (EHSG),

European society of pathology (ESP), and the sociedade Portuguesa de endoscopia digestiva (SPED). Virchows Arch. 2012;460:19–46.

63. Tashiro A, Sano M, Kinameri K, Fujita K, Takeuchi Y. Comparing mass screening techniques for gastric cancer in Japan. World J Gastroenterol. 2006;12:4873–4.
64. Dan YY, So J, Yeoh KG. Endoscopic screening for gastric cancer. Clin Gastroenterol Hepatol. 2006;4:709–16.
65. Morii Y, Arita T, Shimoda K, Yasuda K, Yoshida T, Kitano S. Effect of periodic endoscopy for gastric cancer on early detection and improvement of survival. Gastric Cancer. 2001;4:132–6.
66. Aida K, Yoshikawa H, Mochizuki C, Mori A, Muto S, Fukuda T, et al. Clinicopathological features of gastric cancer detected by endoscopy as part of annual health checkup. J Gastroenterol Hepatol. 2008;23:632–7.
67. Nam SY, Choi IJ, Park KW, Kim CG, Lee JY, Kook MC, et al. Effect of repeated endoscopic screening on the incidence and treatment of gastric cancer in health screenees. Eur J Gastroenterol Hepatol. 2009;21:855–60.
68. Chung SJ, Park MJ, Kang SJ, Kang HY, Chung GE, Kim SG, et al. Effect of annual endoscopic screening on clinicopathologic characteristics and treatment modality of gastric cancer in a high-incidence region of Korea. Int J Cancer. 2012;131:2376–84.

H. pylori Infection-Negative Gastric Cancer

29

Hee Jin Kim

Abstract

Helicobacter pylori (*H. pylori*) infection is considered to be the main etiological factor of gastric carcinogenesis. *H. pylori* infection induces superficial gastritis, which progresses to atrophic gastritis with loss of acid secretion and then to dysplasia and cancer. As *H. pylori* are eliminated once they have caused gastric atrophy and intestinal metaplasia after long-time colonization, the possibility of false-negative results of conventional *H. pylori* tests could increase gastric cancer (GC) with severe gastric atrophy. The incidence of *H. pylori* infection-negative gastric cancer (HPIN-GC) varied among studies according to the definition of *H. pylori*-negative infection. When the combination of multiple methods (histologic examination, rapid urease test, urea breath test, *H. pylori* culturing, anti-*H. pylori* antibody, and polymerase chain reaction assay for *H. pylori*) is used and past *H. pylori* infection is ruled out by the presence of atrophic change, HPIN-GC is compromised by about 0.4–5% of GC. The proximal location is common in HPIN-GC. The more advanced pathologic T stage and N stage in HPIN-GC have been reported in some studies. Many previous studies reported that negative *H. pylori* infection was an independent poor prognostic factor, but some researches found no significant difference in prognosis between *H. pylori*-positive and *H. pylori*-negative cancer.

Keywords

Gastric cancer · *Helicobacter pylori*
Negative infection

29.1 Introduction

Helicobacter pylori (*H. pylori*) is strongly associated with gastric cancer (GC) through epidemiologic and clinical studies, and it has been classified as a class I carcinogen by the International Agency for Research on Cancer [1]. A few prospective cohort studies suggested that GC develops only in *H. pylori* infection-positive subjects [2, 3]. However, no evidence of *H. pylori* infection was found in some patients with GC [4–6], and several studies reported that *H. pylori* infection-negative gastric cancer (HPIN-GC) has distinct clinicopathologic features and prognosis compared with *H. pylori* infection-positive gastric cancer (HPIP-GC), but those studies have not shown the consistent results by various *H. pylori* detection systems that interfered with interpreting the results. This chapter aimed to review the research results on the incidence, clinicopathologic characteristics, and prognosis of HPIN-GC.

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29.2 Definition of *H. pylori* Infection-Negative Gastric Cancer

The definition of *H. pylori*-negative infection has not been consistent in various studies. There are many methods provided for detecting *H. pylori* including histologic examination, rapid urease test, urea breath test, *H. pylori* culturing, and anti-*H. pylori* antibody. Polymerase chain reaction (PCR) assay for *H. pylori* may have been more sensitive compared with other diagnostic techniques. Many studies used a combination of multiple methods for detecting *H. pylori* to minimize the possibility of false-negative *H. pylori* testing results. The methods for definition in HPIN-GC are summarized in Table 29.1.

The diagnostic tests have been challenged because of a natural clearance or an unexpected eradication of *H. pylori* in the gastric mucosa. *H. pylori* spontaneously disappeared in severely atrophic mucosa and advanced gastric diseases once they have caused gastric atrophy and intestinal metaplasia after long-time colonization [15]. Therefore, it is difficult to determine whether patients who had advanced gastric atrophy and intestinal metaplasia have had previous *H. pylori* infection based on conventional *H. pylori* tests. In addition, the spontaneous seroconversion of anti-*H. pylori* antibodies often occurs [16].

To overcome this problem, some studies from Korea and Japan considered atrophic gastritis and intestinal metaplasia as a result of long-standing *H. pylori* infection and determined the presence of severe gastric atrophy or intestinal metaplasia as having a previous *H. pylori* infection. In these studies, the absence of gastric atrophy confirmed by histologic examination and serum pepsinogen (serum pepsinogen I ≤70 ng/mL and/or pepsinogen I/II ratio ≤3.0) was also added to define HPIN-GC [4, 6, 8, 10, 11].

A previous Japanese review [17] on the characteristics of HPIN-GC defined a minimum set of criteria for definition, that is, negative findings in two or more methods including endoscopic or pathologic findings or serum pepsinogen test, a negative urea breath test or serum IgG test, and no history of *H. pylori* eradication. Lack of mucosal atrophy and presence of regular arrangement of collecting venules on the mucosal surface were added for endoscopic diagnosis. For pathologic diagnosis, non-atrophic mucosa without *H. pylori* infection is graded with regard to glandular atrophy (0), intestinal metaplasia (0 or 1), mononuclear cells (0), neutrophils (0), and *H. pylori* density (0) using the Updated Sydney classification. The authors also recommended stricter criteria that require assessment by endoscopic, pathologic, as well as two or more *H. pylori* tests (e.g., rapid urease test, urease breath test, serum IgG, or stool antigen), a serum pepsinogen test, and determination of *H. pylori* eradication history.

Most recent Japanese studies on HPIN-GC [5, 11, 17] used strict criteria to define the HPIN-GC and pathologically classify HPIN-GC into several types. Yamamoto et al. [17] reported the characteristics of 16 cases with HPIN-GC and classified it as the undifferentiated type that was mainly pure signet-ring cell carcinoma, fundic gland type, and foveolar type. Mizutani et al. [18] reported 19 cases with HPIN-GC and classified them into undifferentiated type, fundic gland type, foveolar epithelial type, cardiac gland type, pyloric gland type, and mixed type GC.

Table 29.1 Clinicopathologic characteristics of *H. pylori* infection-negative gastric cancer

Author (publication year)	Duration of study	Country	Number of total subjects	Number of *H. pylori* infection-negative gastric cancer patients (%)	Definition of *H. pylori* infection-negative gastric cancer	Characteristics of *H. pylori* infection-negative gastric cancer
Tsai et al. (2017) [7]	1998–2011	Taiwan	567	75 (13.2)	Negative results of histology and serology No history of *H. pylori* eradication	Younger, proximal location, diffuse-type, more stage IV, poor overall survival
					Absence of serologic atrophy (serum pepsinogen I <70 ng/mL and serum pepsinogen I/II ratio ≤3)	
Kim et al. (2015) [8]	2006–2014	Korea	705	28 (4.0)	Negative results of histology, serology, culture, and rapid urease test No history of *H. pylori* eradication	–
					Absence of histologic atrophy and serologic atrophy (serum pepsinogen I/II ratio ≤3)	
Kwak et al. (2014) [9]	2007–2012	Korea	1833	43 (2.3)	Negative results of histology, serology, and rapid urease test	Younger, female, diffuse-type, distant metastasis[a]
					Absence of histologic atrophy	
Kim et al. (2014) [10]	2003–2012	Korea	726	36 (5.0)	Negative results of histology, serology, culture, and rapid urease test No history of *H. pylori* eradication	Cardia location
					Absence of histologic atrophy and serologic atrophy (serum pepsinogen I/II ratio ≤3)	
Ono et al. (2012) [11]	2004–2010	Japan	240	1 (0.42)	Negative results of histology, serology, culture, rapid urea test, and/or urea breath test No history of *H. pylori* eradication	–
					Absence of histologic atrophy, endoscopic atrophy, and serologic atrophy (serum pepsinogen I <70 ng/mL and/or pepsinogen I/ II ratio <3.0)	

(continued)

Table 29.1 (continued)

Author (publication year)	Duration of study	Country	Number of total subjects	Number of *H. pylori* infection-negative gastric cancer patients (%)	Definition of *H. pylori* infection-negative gastric cancer	Characteristics of *H. pylori* infection-negative gastric cancer
Yoon et al. (2011) [4]	2003–2010	Korea	627	34 (5.4)	Negative results of histology, serology, culture, and rapid urease test No history of *H. pylori* eradication	Advanced pT stage
					Absence of histologic atrophy and serologic atrophy (serum pepsinogen <30 ng/mL and/or pepsinogen I/II ratio <2.0)	
Matsuo et al. (2011) [5]	1996–2010	Japan	3161	21 (0.66)	Negative results of histology, serology, rapid urease test and/or urea breath test	Younger, female, diffuse type, macroscopic depressed lesion
					Absence of endoscopic atrophy and histologic gastritis	
Kakinoki et al. (2009) [12]	2004–2007	Japan	386	12 (3.1)	Negative histology	Upper location, poorly differentiated carcinoma
Marrelli et al. (2009) [13]	1988–2004	Italy	297	41 (13.8)	Negative results of serology, PCR assay for *vacA*^{b} gene and CagA^c antibody	Cardia location, advanced pT stage, non-curative surgery, lower 5-year survival rate
Kato et al. (2007) [6]	1993–2004	Japan	748	15 (2–10.6)	Negative serology No history of *H. pylori* eradication	–
					Absence of serologic atrophy (serum pepsinogen <30 ng/mL and/or pepsinogen I/II ratio <2.0)	
Meimarakis et al. (2006) [14]	1992–2002	Germany	166	41 (24.6)	Negative results of histology, serology, and culture No history of *H. pylori* eradication	Upper location, lower relapse-free, and overall survival

[a] Compared to gastric cancer with past *H. pylori* infection

[b] *vacA* vacuolating cytotoxin

[c] *CagA* a product of the cytotoxin-associated gene pathogenicity island

29.3 Incidence

The incidence of HPIN-GC varied among studies. Differences in the definition of *H. pylori*-negative infection may partly explain the differences in various studies. Results of retrospective studies conducted in Korea have shown that HPIN-GC comprised 2.3–5.4% of GCs [4, 8–10]. Japanese studies also showed that the incidence of HPIN-GC ranged from 0.42 to 10.6% [5, 6, 11, 12]. In contrast, the proportions of HPIN-GC in Western countries, such as Italy [13] and Germany [14], ranged from 13.8 to 24.6%. The results of previous studies on HPIN-GC are summarized in Table 29.1.

The Stomach Cancer Pooling (StoP) Project, a consortium of case–control and nested case–control studies, which uses an individual participant data approach for the evaluation of the associations between risk factors and GC, has reported that the prevalence of HPIN-GC was 6.6%, following the reclassification of serology-defined negative *H. pylori* infection status as positive when cases presented either anti-cytotoxin-associated gene A (CagA) antibodies, gastric atrophy, or advanced stage at diagnosis [19]. CagA serostatus independently of *H. pylori* infection status has been used as a more sensitive marker of past infection in previous researches [20, 21].

The Western studies did not consider atrophic mucosal changes or intestinal metaplasia. In contrast, studies from Korea and Japan usually consider atrophic change as evidence of previous *H. pylori* infection. Therefore, studies from Eastern Asia report a very low incidence of HPIN-GC compared with studies from Western countries. In conclusion, HPIN-GC is compromised about 2–5% of GC, when negative *H. pylori* infection was established by the combination of various methods for detecting *H. pylori* and when the absence of atrophic gastritis was confirmed by serum pepsinogen and histologic examination. When endoscopic and pathologic diagnoses were added to the definition of HPIN-GC, HPIN-GC consists of less than 1% of GC.

Improvement in sanitary environment and widespread *H. pylori* eradication therapy have led to a decrease in *H. pylori* infection in Korea and Japan. Therefore, the incidence of GC decreases and the incidence of HPIN-GC shows a relatively increase in the future.

29.4 Clinicopathologic Characteristics

In the literature, the higher distributions of the cardia or upper location were frequently reported in HPIN-GC [7, 10, 12–14, 22, 23]. The histologically diffuse type and female predominance in HPIN-GC were also found in some researches [5, 9, 22]. With regard to the pathologic T and N staging, the results were not consistent. Some studies reported the more advanced pathologic T stage [4, 13], N stage [23], and IV stage [7] in HPIN-GC compared to HPIP-GC, but some showed that the stage of HPIN-GC was not significantly different from that of HPIP-GC [8, 10, 14]. In a Korean study, HPIN-GC did not have different characteristics compared to HPIP-GC with regard to well-known independent risk factors of GC such as age, gender, and family history of GC [8].

Japanese studies reported the characteristics of subtypes of HPIN-GC. Signet-ring cell carcinoma is the most common type, which is younger patient age, a discolored lesion, frequently identified in the middle or lower third of the stomach, localization to the mucosal proliferation zone, smaller tumor size, and lower Ki-6 labeling index [17, 18, 24]. Gastric adenocarcinoma of fundic gland type is a new rare variant of gastric adenocarcinoma and presents with atypical cells with differentiation toward the fundic gland, which are positive for immunohistochemical staining of pepsinogen I (a marker of chief cells) and/or H^+K^+-ATPase (a marker of parietal cells). It was newly listed as rare subtypes in the fifth edition of the World Health Organization classification of digestive system tumors published in 2019 [25]. It often occurs in the upper to middle third of the stomach and appears endoscopically in two types such as submucosal tumor shape and flat or depressed [17, 18, 26]. Other differentiated gastric adenocarcinoma of HPIN-GC is rare, and various types have been proposed to date [27]. However, with the small number of patients in these studies, it is difficult to identify the consistent clinicopathological features of HPIN-GC.

29.5 Molecular Characteristics

Recently, Kim et al. compared the p53 overexpression and microsatellite instability (MSI) between HPIN-GC and HPIP-GC in their two researches, and they showed that there was no significant difference between the two groups with regard to the p53 overexpression and MSI [8, 10].

29.6 Prognosis

The impact of *H. pylori* infection on the survival of GC is poorly investigated. A few studies reported the association between negative *H. pylori* infection and clinical outcomes, and the results on the prognosis of HPIN-GC were not consistent among the studies [14, 17, 18, 27–29].

Meimarakis et al. assessed *H. pylori* infection status of 166 GC patients who had curative resection using bacterial culture, histological analyses, and serological analyses [14]. In this study conducted in Germany, the overall survival was 19.2 months (range, 7.1–31.3) in 41 HPIN-GC patients and 61.9 months (range, 13.0–110.9) in 125 HPIP-GC patients ($p = 0.0009$), and the positive *H. pylori* infection was an independent prognostic factor for overall survival (hazard ratio [HR] 2.16; 95% confidential interval [CI], 1.33–3.49) in addition to depth of invasion, lymph node metastasis, and age.

In another study conducted in Italy, in which *H. pylori* status was determined by serologic assay of *H. pylori* and CagA antibodies and PCR assay for *vacA* gene in gastric mucosa, the 5-year survival rate was 24% in 41 HPIN-GC patients and 57% in 256 HPIP-GC patients (log-rank test, $p < 0.001$) [13]. *H. pylori* infection, depth of invasion, lymph node metastasis, and tumor location were reported to be independent prognostic factors, and the negative *H. pylori* infection was poor prognostic factor in the early (pT1–pT2) and in the more advanced pT classification groups (pT3–pT4). Two Korean studies reported similar results that negative *H. pylori* status was the significant independent poor prognostic factor and determined *H. pylori* infection status of GC patients who underwent chemotherapy using only histologic examination [28, 29]. Taiwan study defined the HPIN-GC as negative results of serology and histology and absence of serologic atrophy using serum pepsinogen and reported that the long-term overall survival rate was 35.8% in HPIP-GC patients and 24.0% in HPIN-GC patients ($p = 0.035$) [7]. Negative *H. pylori* infection status was an independent prognostic factor for poor overall survival (HR 1.34; 95% CI, 1.04–1.7, $p = 0.019$) in this study [7]. Also, the meta-analysis involving a total of 12 studies including 2454 patients with GC concluded a protective role for *H. pylori* infection in the prognosis of GC [30].

However, Kim et al. found no significant difference in prognosis between HPIN-GC and HPIP-GC, defining HPIN-GC strictly as a GC having all negative results for *H. pylori* tests and absence of histologic atrophy and serologic atrophy using pepsinogen [8]. In this study, the 5-year rate of overall survival was 93.8% in 24 HPIN-GC patients and 96.9% in 509 HPIP-GC patients ($p = 0.685$); the status of *H. pylori* infection was not an independent prognostic factor for overall survival (HR 0.51; 95% CI, 0.07–3.70). Similarly, the study conducted in China reported that overall survival and relapse-free survival in HPIN-GC were not significantly different from HPIP-GC patients ($p = 0.715$), assessing *H. pylori* infection status using PCR assay for *H. pylori* [23].

Some authors suggest that *H. pylori* infection could improve immune responses against GC, leading to improved survival of patients with positive *H. pylori* status [14]. However, several investigators doubt the true prognostic significance of *H. pylori* status, suggesting that negative *H. pylori* status may be simply related to more advanced tumor progression such as cardia location and more advanced stage [31]. Further studies will be required to evaluate these hypotheses from the perspective of molecular and genetic aspects.

29.7 Conclusions

GCs that are not associated with *H. pylori* infection are rare. HPIN-GC comprised 0.42–5.4% of GCs when past *H. pylori* infection was excluded by the presence of atrophic change. Clinicopathological features have not been fully documented yet. Proximal location is common in HPIN-GC. The prognosis of HPIN-GC has not been fully established. Therefore, further study recruiting a large number of patients for a long time and defining negative *H. pylori* infection precisely is needed to understand the exact carcinogenesis of HPIN-GC.

References

1. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process—first American Cancer Society award lecture on cancer epidemiology and prevention. Cancer Res. 1992;52:6735–40.
2. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, et al. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med. 2001;345:784–9.
3. Ohata H, Kitauchi S, Yoshimura N, Mugitani K, Iwane M, Nakamura H, et al. Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer. Int J Cancer. 2004;109:138–43.
4. Yoon H, Kim N, Lee HS, Shin CM, Park YS, Lee DH, et al. *Helicobacter pylori*-negative gastric cancer in South Korea: incidence and clinicopathologic characteristics. Helicobacter. 2011;16:382–8.
5. Matsuo T, Ito M, Takata S, Tanaka S, Yoshihara M, Chayama K. Low prevalence of *Helicobacter pylori*-negative gastric cancer among Japanese. Helicobacter. 2011;16:415–9.
6. Kato S, Matsukura N, Tsukada K, Matsuda N, Mizoshita T, Tsukamoto T, et al. *Helicobacter pylori* infection-negative gastric cancer in Japanese hospital patients: incidence and pathological characteristics. Cancer Sci. 2007;98:790–4.
7. Tsai KF, Liou JM, Chen MJ, Chen CC, Kuo SH, Lai IR, et al. Distinct clinicopathological features and prognosis of *Helicobacter pylori* negative gastric cancer. PLoS One. 2017;12:e0170942.
8. Kim HJ, Kim N, Yoon H, Choi YJ, Lee JY, Kwon YH, et al. Comparison between resectable *Helicobacter pylori*-negative and -positive gastric cancers. Gut Liver. 2016;10:212–9.
9. Kwak HW, Choi IJ, Cho SJ, Lee JY, Kim CG, Kook MC, et al. Characteristics of gastric cancer according to *Helicobacter pylori* infection status. J Gastroenterol Hepatol. 2014;29:1671–7.
10. Kim HJ, Hwang SW, Kim N, Yoon H, Shin CM, Park YS, et al. *Helicobacter pylori* and molecular markers as prognostic indicators for gastric cancer in Korea. J Cancer Prev. 2014;19:56–67.
11. Ono S, Kato M, Suzuki M, Ishigaki S, Takahashi M, Haneda M, et al. Frequency of *Helicobacter pylori*-negative gastric cancer and gastric mucosal atrophy in a Japanese endoscopic submucosal dissection series including histological, endoscopic and serological atrophy. Digestion. 2012;86:59–65.
12. Kakinoki R, Kushima R, Matsubara A, Saito Y, Okabe H, Fujiyama Y, et al. Re-evaluation of histogenesis of gastric carcinomas: a comparative histopathological study between *Helicobacter pylori*-negative and *H. pylori*-positive cases. Dig Dis Sci. 2009;54:614–20.
13. Marrelli D, Pedrazzani C, Berardi A, Corso G, Neri A, Garosi L, et al. Negative *Helicobacter pylori* status is associated with poor prognosis in patients with gastric cancer. Cancer. 2009;115:2071–80.
14. Meimarakis G, Winter H, Assmann I, Kopp R, Lehn N, Kist M, et al. *Helicobacter pylori* as a prognostic indicator after curative resection of gastric carcinoma: a prospective study. Lancet Oncol. 2006;7:211–22.
15. Kang HY, Kim N, Park YS, Hwang JH, Kim JW, Jeong SH, et al. Progression of atrophic gastritis and intestinal metaplasia drives *Helicobacter pylori* out of the gastric mucosa. Dig Dis Sci. 2006;51:2310–5.
16. Kokkola A, Kosunen TU, Puolakkainen P, Sipponen P, Harkonen M, Laxen F, et al. Spontaneous disappearance of *Helicobacter pylori* antibodies in patients with advanced atrophic corpus gastritis. APMIS. 2003;111:619–24.
17. Yamamoto Y, Fujisaki J, Omae M, Hirasawa T, Igarashi M. *Helicobacter pylori*-negative gastric cancer: characteristics and endoscopic findings. Dig Endosc. 2015;27:551–61.
18. Mizutani T, Araki H, Saigo C, Takada J, Kubota M, Ibuka T, et al. Endoscopic and pathological characteristics of *Helicobacter pylori* infection-negative early gastric cancer. Dig Dis. 2020;38:474–83.
19. Morais S, Peleteiro B, Araújo N, et al. Identifying the profile of *Helicobacter pylori* negative gastric cancers: a case-only analysis within the stomach cancer pooling (StoP) project. Cancer Epidemiol Biomark Prev. 2022;31:200–9.
20. Peleteiro B, Lunet N, Barros R, La Vecchia C, Barros H. Factors contributing to the underestimation of *Helicobacter pylori*-associated gastric cancer risk in a high-prevalence population. Cancer Causes Control. 2010;21:1257–64.
21. Ekstrom AM, Held M, Hansson LE, Engstrand L, Nyren O. *Helicobacter pylori* in gastric cancer estab-

lished by CagA immunoblot as a marker of past infection. Gastroenterology. 2001;121:784–91.

22. Wu MS, Hung HW, Wang JT, Tseng CC, Shun CT, Wang HP, et al. *Helicobacter pylori*-seronegative gastric carcinoma: a subset of gastric carcinoma with distinct clinicopathologic features. Hepato-Gastroenterology. 1998;45:2432–6.
23. Qiu HB, Zhang LY, Keshari RP, Wang GQ, Zhou ZW, Xu DZ, et al. Relationship between *H. pylori* infection and clinicopathological features and prognosis of gastric cancer. BMC Cancer. 2010;10:374.
24. Horiuchi Y, Fujisaki J, Ishizuka N, Omae M, Ishiyama A, Yoshio T, et al. Study on clinical factors involved in *Helicobacter pylori*-uninfected, undifferentiated-type early gastric cancer. Digestion. 2017;96:213–9.
25. Carneiro FFM, Grabsch HI, Yasui W. Tumours of the stomach. World Health Organization classification of tumours editorial board. Lyon: IARC Press; 2019. p. 92.
26. Ueyama H, Matsumoto K, Nagahara A, Hayashi T, Yao T, Watanabe S. Gastric adenocarcinoma of fundic gland type (chief cell predominant type): proposal for a new entity of gastric adenocarcinoma. Am J Surg Pathol. 2010;34:609–19.
27. Yamada A, Kaise M, Inoshita N, Toba T, Nomura K, Kuribayashi Y, et al. Characterization of *Helicobacter pylori*-naïve early gastric cancers. Digestion. 2018;98:127–34.
28. Choi IK, Sung HJ, Lee JH, Kim JS, Seo JH. The relationship between *Helicobacter pylori* infection and the effects of chemotherapy in patients with advanced or metastatic gastric cancer. Cancer Chemother Pharmacol. 2012;70:555–8.
29. Kang SY, Han JH, Ahn MS, Lee HW, Jeong SH, Park JS, et al. *Helicobacter pylori* infection as an independent prognostic factor for locally advanced gastric cancer patients treated with adjuvant chemotherapy after curative resection. Int J Cancer. 2012;130:948–58.
30. Wang F, Sun G, Zou Y, Zhong F, Ma T, Li X. Protective role of *Helicobacter pylori* infection in prognosis of gastric cancer: evidence from 2454 patients with gastric cancer. PLoS One. 2013;8:e62440.
31. Rad R, Prinz C, Schmid RM. *Helicobacter pylori* and prognosis of gastric carcinoma. Lancet Oncol. 2006;7:364–5.

Gastroesophageal Reflux Disease

30

Jinjoo Kim

Abstract

Helicobacter pylori (*H. pylori*) infection and its eradication can influence gastroesophageal reflux disease (GERD) through various mechanisms, including alterations in gastric acid secretion and inflammatory responses. The relationship between gastric acid secretion and *H. pylori* infection involves several proposed mechanisms, such as somatostatin secretion by D cells in the gastric antrum, feedback inhibition on gastrin release, and changes in cytokine levels impacting local inflammation severity. The impact of *H. pylori* infection on GERD can be vary depending on the affected areas' distribution, leading to either attenuation or exacerbation. However, the potential effects of *H. pylori* eradication on GERD should not deter treatment efforts. Over the long term, *H. pylori* eradication alone does not necessarily contribute to GERD development but may correlates with other diseases.

Keywords

Helicobacter pylori · Gastroesophageal reflux disease · Inflammation · Gastric acid secretion

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30.1 Introduction

The relationship between gastroesophageal reflux disease (GERD) and *Helicobacter pylori* (*H. pylori*) presents a complexity distinct from that observed in peptic ulcer disease, gastric adenocarcinoma, and primary B cell lymphoma of the stomach. Various epidemiological studies have highlighted an increased incidence of GERD and esophageal adenocarcinoma alongside a decrease in *H. pylori* prevalence [1, 2]. Consequently, numerous researchers have investigated the connections between *H. pylori* and GERD. This chapter aims to delve into the epidemiology and pathophysiology of *H. pylori* and GERD, along with exploring the potential role of *H. pylori* eradication in GERD management.

30.2 Epidemiology of GERD in *H. pylori*-Infected Population

Several studies have indicated that individuals with *H. pylori* infection are less prone to developing GERD, and among GERD patients, those with *H. pylori* infection often exhibit less severe esophagitis compared to their *H. pylori*-negative counterparts [3–7]. Furthermore, certain studies propose that colonization with *H. pylori* CagA strains, recognized for their increased virulence and stronger correlation with gastric adenocarcinoma, might confer protection against the development of severe GERD [8, 9].

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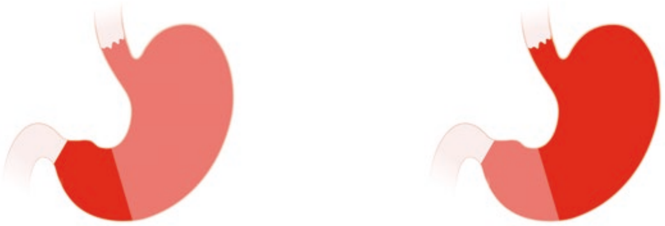
30.3 The Impact of *H. pylori* Infections on GERD

Reflux of gastric contents into the esophagus is a common occurrence even among healthy individuals and often goes unnoticed. However, when certain barriers such as the lower esophageal sphincter (LES), the surrounding diaphragm, the location of the LES, the phrenoesophageal ligament, and the acute angle of His become compromised, these refluxes can manifest in various symptoms [10–12]. Factors such as transient LES relaxation, hiatal hernia, and low LES pressure play crucial roles in this process, with *H. pylori* itself not directly influencing these barriers [13–17].

It is hypothesized that *H. pylori* exerts its influence on gastric acid secretion, thereby potentially contributing to the onset of GERD. The secretion of gastric acid is modulated by *H. pylori*-induced inflammation, which varies depending on its distribution [18–20]. Upon food entery into the stomach, gastric acid secretion is triggered, regulated by feedback reaction to gastric pH levels. Typically, food indigestion results in a rise in stomach pH, prompting gastric acid secretion followed by pH reduction. However, the presence of undigested food proteins can sustain higher pH levels, stimulating further gastric acid secretion by the stomach's parietal cells. This process triggers somatostatin secretion, inhibiting additional gastric acid secretion. Yet, the presence of an *H. pylori* infection can disrupt this feedback loop, altering gastric acid secretion regulation [21].

As depicted in Fig. 30.1 [22], antral-predominant gastritis inhibits somatostatin secretion in the antrum, leading to inadequate inhibition of gastric acid and elevated acid levels [23]. Conversely, corpus-predominant gastritis results in damage to the corpus's parietal cells, causing decreased acid secretion and no feedback inhibition [24–26].



	Antral-predominant gastritis	Corpus-predominant gastritis
Acid secretion	↑	↓
Rick of disease development	Duodenal ulcer (×10)	Stomach cancer (×2–3)
	After *H. pylori* eradication	
GERD(+)	Improved	Improved
GERD(−)	No significant change	Might develop GERD

Fig. 30.1 Relationship between gastroesophageal reflux disease (GERD) and *H. pylori* (modified from Pandolfino and Kahrilas [22])

30.4 Influence of *H. pylori* Eradication on GERD

Clinical experience with some patients developing GERD symptoms after *H. pylori* eradication has promoted studies of the effect of *H. pylori* eradication on the development of GERD [27, 28]. How *H. pylori* eradication affects GERD depends on the levels of gastric acid secretion and the physiological barriers to prevent GERD that have worked before the eradication. Also, the proton pump inhibitor (PPI) rebound phenomenon might be one of the reasons, even though the duration of PPI usage as *H. pylori* eradication is quite short.

According to several studies, when GERD patients were followed up after *H. pylori* eradication, for 6 months to 1 year, their symptoms and endoscopic findings were not generally aggravated [29, 30]. However, the studies on the actual pH change of the distal esophagus are scant and controversial yet, probably because it is not easy to measure the distal esophageal pH [31–35]. There are some discrepancies in the studies about the relation between *H. pylori* eradication and GERD. This might be explained that initial studies could not consider that different *H. pylori*-associated inflammation distributions would result in different outcomes. Taking this limitation into account, the results of the studies so far can be summarized as follows.

30.4.1 Antral-Predominant Gastritis

Antral-predominant gastritis is a condition that increases gastric acid secretion, putting patients at high risk for developing gastroesophageal reflux disease (GERD). Fortunately, eradicating *H. pylori* infection can decrease the risk of developing GERD [28, 36–40]. In fact, studies have shown that *H. pylori* eradication can improve GERD symptoms in patients with duodenal ulcer and/or GERD. For example, eight studies involving 1165 duodenal ulcer patients found that *H. pylori* eradication did not lead to the development of new GERD symptoms and improved preexisting GERD symptoms [39]. Furthermore, a meta-analysis of 27 studies found no evidence of GERD symptoms developing after *H. pylori* eradication in patients with duodenal ulcer [40].

30.4.2 Corpus-Predominant Gastritis

Corpus-predominant gastritis is characterized by reduced gastric acid secretion, which typically prevents the occurrence of GERD. However, when *H. pylori* is eradicated in patients with corpus-predominant gastritis, their condition may improve, and acid secretion may increase, leading to the development of GERD. This concept is supported by several studies that have demonstrated that individuals with severe corpus gastritis are less likely to experience GERD symptoms, and the improvement of this gastritis can increase the risk of GERD development [41–44].

It is important to note that the eradication of *H. pylori* does not directly cause GERD. Rather, it unmasks the symptoms in patients who have weak GERD barriers. Patients who are unable to clear the acid in the esophagus and have hiatal hernias or low LES pressure are at a higher risk of developing GERD, a risk that has been masked by *H. pylori*-associated inflammation for some time. This concept can be depicted as an “iceberg of GERD,” as shown in Fig. 30.2 [18].

The risk of developing GERD after *H. pylori* eradication was analyzed in a study that showed that patients with hiatal hernias, a known risk factor for GERD, had a higher incidence of reflux esophagitis after *H. pylori* eradication (26% compared to 8%). In other words, patients with higher risk factors for GERD may be more likely to develop GERD symptoms, whereas those without such factors may not.

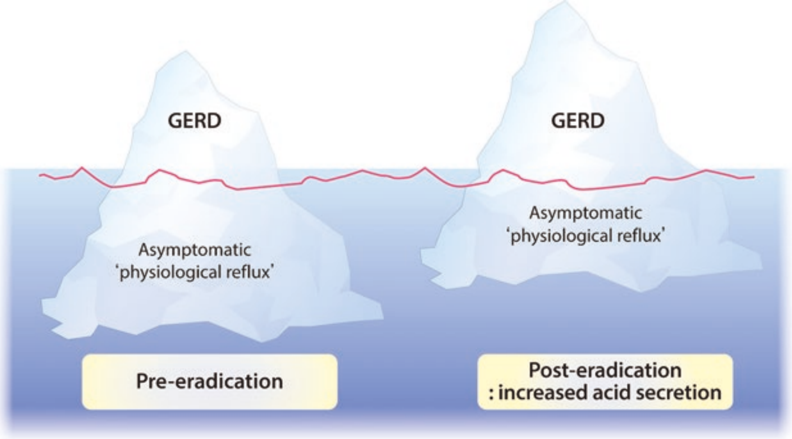


Fig. 30.2 Iceberg of gastroesophageal reflux disease (modified from Graham [18])

30.5 Conclusions

In conclusion, while *H. pylori* can influence gastric acid secretion and potentially contribute to the development of GERD in certain patients, this risk does not outweigh the benefits of eradicating *H. pylori*. Therefore, there is no need to hesitate to undergo *H. pylori* eradication due to concerns about this issue.

References

1. Hackelsberger A, Schultze V, Gunther T, von Arnim U, Manes G, Malfertheiner P. *Helicobacter pylori* prevalence in reflux esophagitis: a case control study. Eur J Gastroenterol Hepatol. 1997;10:465–8.
2. El-Serag HB, Sonnenberg A. Opposing time trends of peptic ulcer and reflux disease. Gut. 1998;43:327–33.
3. Kandulski A, Malfertheiner P. *Helicobacter pylori* and gastroesophageal reflux disease. Curr Opin Gastroenterol. 2014;30:402–7.
4. Shirota T, Kusano M, Kawamura O, Horikoshi T, Mori M, Sekiguchi T. *Helicobacter pylori* infection correlates with severity of reflux esophagitis: with manometry findings. J Gastroenterol. 1999;34:553–9.
5. Chung SJ, Lim SH, Choi J, Kim D, Kim YS, Park MJ, et al. *Helicobacter pylori* serology inversely correlated with the risk and severity of reflux esophagitis in *Helicobacter pylori* endemic area: a matched case-control study of 5616 health check-up Koreans. J Neurogastroenterol Motil. 2011;17:267–73.
6. Weston AP, Badr AS, Topalovski M, Cherian R, Dixon A, Hassanein RS. Prospective evaluation of the prevalence of gastric *Helicobacter pylori* infection in patients with GERD, Barrett's esophagus, Barrett's dysplasia, and Barrett's adenocarcinoma. Am J Gastroenterol. 2000;95:387–94.
7. Nam SY, Choi IJ, Ryu KH, Kim BC, Kim CG, Nam BH. Effect of *Helicobacter pylori* infection and its eradication on reflux esophagitis and reflux symptoms. Am J Gastroenterol. 2010;105:2153–62.
8. Vaezi MF, Falk GW, Peek RM, Vicari JJ, Goldblum JR, Perez-Perez GI, et al. CagA-positive strains of *Helicobacter pylori* may protect against Barrett's esophagus. Am J Gastroenterol. 2000;95:2206–11.
9. Vicari JJ, Peek RM, Falk GW, Goldblum JR, Easley KA, Schnell J, et al. The seroprevalence of CagA-positive *Helicobacter pylori* strains in the spectrum of gastroesophageal reflux disease. Gastroenterology. 1998;115:50–7.
10. Ellis FH Jr, Lyons WS, Olsen AM. The gastroesophageal sphincter mechanism: a review. Proc Staff Meet Mayo Clin. 1956;31:605–14.
11. Hill LD, Kozarek RA. The gastroesophageal flap valve. J Clin Gastroenterol. 1999;28:194–7.
12. Hill LD, Kozarek RA, Kraemer SJ, Aye RW, Mercer CD, Low DE, et al. The gastroesophageal flap valve: in vitro and in vivo observations. Gastrointest Endosc. 1996;44:541–7.
13. Wong WM, Lai KC, Hui WM, Hu WH, Huang JQ, Wong NY, et al. Pathophysiology of gastroesophageal reflux diseases in Chinese-role of transient lower esophageal sphincter relaxation and esophageal motor dysfunction. Am J Gastroenterol. 2004;99:2088–93.
14. Zerbib F, Bicheler V, Leray V, Joubert M, Bruley Des Varannes S, Galmiche JP. *H. pylori* and transient lower esophageal sphincter relaxations induced by gastric distension in healthy humans. Am J Physiol Gastrointest Liver Physiol. 2001;281:G350–6.
15. Tanaka I, Tatsumi Y, Kodama T, Kato K, Fujita S, Mitsufuji S, et al. Effect of *Helicobacter pylori* eradication on gastroesophageal function. J Gastroenterol Hepatol. 2004;19:251–7.
16. Graham DY. *Campylobacter pylori* and Barrett's esophagus. Mayo Clin Proc. 1988;63:1258–60.

17. Rugge M, Russo V, Busatto G, Genta RM, Di Mario F, Farinati F, et al. The phenotype of gastric mucosa coexisting with Barrett's oesophagus. J Clin Pathol. 2001;54:456–60.
18. Graham DY. The changing epidemiology of GERD: geography and *Helicobacter pylori*. Am J Gastroenterol. 2003;98:1462–70.
19. Graham DY. *Helicobacter pylori* and perturbations in acid secretion: the end of the beginning. Gastroenterology. 1996;110:1647–50.
20. Dore MP, Graham DY. Ulcers and gastritis. Endoscopy. 2004;36:42–7.
21. Liu Y, Vosmaer GD, Tytgat GN, Xiao SD, Ten Kate FJ. Gastrin (G) cells and somatostatin (D) cells in patients with dyspeptic symptoms: *Helicobacter pylori* associated and non-associated gastritis. J Clin Pathol. 2005;58:927–31.
22. Pandolfino JE, Kahrilas PJ. *Helicobacter pylori* and gastroesophageal reflux disease. In: UpToDate 2016. http://www.uptodate.com/contents/image?imageKey=GAST/53651&topicKey=GAST%2F11&source=outline_link&search=GERD+helicobacter&selectedTitle=1~150&utdP. Accessed 23 Feb 2023.
23. El-Omar EM, Penman ID, Ardill JE, Chittajallu RS, Howie C, McColl KE. *Helicobacter pylori* infection and abnormalities of acid secretion in patients with duodenal ulcer disease. Gastroenterology. 1995;109:681–91.
24. Feldman M, Cryer B, Sammer D, Lee E, Spechler SJ. Influence of *H. pylori* infection on meal-stimulated gastric acid secretion and gastroesophageal acid reflux. Am J Phys. 1999;277:G1159–64.
25. Graham DY, Asaka M. Eradication of gastric cancer and more efficient gastric cancer surveillance in Japan: two peas in a pod. J Gastroenterol. 2010;45:1–8.
26. Comfort MW. Gastric acidity before and after development of gastric cancer: its etiologic, diagnostic and prognostic significance. Ann Intern Med. 1951;34:1331–48.
27. Schütze K, Hentschel E, Dragosics B, Hirschl AM. *Helicobacter pylori* reinfection with identical organisms: transmission by the patients' spouses. Gut. 1995;36:831–3.
28. Labenz J, Blum AL, Bayerdörffer E, Meining A, Stolte M, Börsch G. Curing *Helicobacter pylori* infection in patients with duodenal ulcer may provoke reflux esophagitis. Gastroenterology. 1997;112:1442–7.
29. Raghunath A, Hungin AP, Wooff D, Childs S. Prevalence of *Helicobacter pylori* in patients with gastro-oesophageal reflux disease: systematic review. BMJ. 2003;326:737.
30. Qian B, Ma S, Shang L, Qian J, Zhang G. Effects of *Helicobacter pylori* eradication on gastroesophageal reflux disease. Helicobacter. 2011;16:255–65.
31. Moayyedi P, Bardhan C, Young L, Dixon MF, Brown L, Axon AT. *Helicobacter pylori* eradication does not exacerbate reflux symptoms in gastroesophageal reflux disease. Gastroenterology. 2001;121:1120–6.
32. Schwizer W, Thumshirn M, Dent J, Guldenschuh I, Menne D, Cathomas G, et al. *Helicobacter pylori* and symptomatic relapse of gastro-oesophageal reflux disease: a randomised controlled trial. Lancet. 2001;357:1738–42.
33. Gillen D, Wirz AA, Ardill JE, McColl KE. Rebound hypersecretion after omeprazole and its relation to on-treatment acid suppression and *Helicobacter pylori* status. Gastroenterology. 1999;116:239–47.
34. Reimer C, Søndergaard B, Hilsted L, Bytzer P. Proton-pump inhibitor therapy induces acid-related symptoms in healthy volunteers after withdrawal of therapy. Gastroenterology. 2009;137:80–7.
35. McColl KE, Gillen D. Evidence that proton-pump inhibitor therapy induces the symptoms it is used to treat. Gastroenterology. 2009;137:20–2.
36. McColl KE, Dickson A, El-Nujumi A, El-Omar E, Kelman A. Symptomatic benefit 1–3 years after *H. pylori* eradication in ulcer patients: impact of gastroesophageal reflux disease. Am J Gastroenterol. 2000;95:101–5.
37. Vakil N, Hahn B, McSorley D. Recurrent symptoms and gastro-oesophageal reflux disease in patients with duodenal ulcer treated for *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2000;14:45–51.
38. Ishiki K, Mizuno M, Take S, Nagahara Y, Yoshida T, Yamamoto K, et al. *Helicobacter pylori* eradication improves pre-existing reflux esophagitis in patients with duodenal ulcer disease. Clin Gastroenterol Hepatol. 2004;2:474–9.
39. Laine L, Sugg J. Effect of *Helicobacter pylori* eradication on development of erosive esophagitis and gastroesophageal reflux disease symptoms: a post hoc analysis of eight double blind prospective studies. Am J Gastroenterol. 2002;97:2992–7.
40. Raghunath AS, Hungin AP, Wooff D, Childs S. Systematic review: the effect of *Helicobacter pylori* and its eradication on gastro-oesophageal reflux disease in patients with duodenal ulcers or reflux oesophagitis. Aliment Pharmacol Ther. 2004;20:733–44.
41. Wu JC, Sung JJ, Chan FK, Ching JY, Ng AC, Go MY, et al. *Helicobacter pylori* infection is associated with milder gastro-oesophageal reflux disease. Aliment Pharmacol Ther. 2000;14:427–32.
42. Koike T, Ohara S, Sekine H, Iijima K, Abe Y, Kato K, et al. *Helicobacter pylori* infection prevents erosive reflux oesophagitis by decreasing gastric acid secretion. Gut. 2001;49:330–4.
43. Hamada H, Haruma K, Mihara M, Kamada T, Yoshihara M, Sumii K, et al. High incidence of reflux oesophagitis after eradication therapy for *Helicobacter pylori*: impacts of hiatal hernia and corpus gastritis. Aliment Pharmacol Ther. 2000;14:729–35.
44. Iijima K, Ohara S, Sekine H, Koike T, Kato K, Asaki S, et al. Changes in gastric acid secretion assayed by endoscopic gastrin test before and after *Helicobacter pylori* eradication. Gut. 2000;46:20–6.

31

NSAID-Induced Gastropathy and *H. pylori* Infection

Gwang Ha Kim

Abstract

Gastrointestinal (GI) injury caused by nonsteroidal anti-inflammatory drugs (NSAIDs) or *Helicobacter pylori* (*H. pylori*) occurs via different mechanisms. If these two factors coexist, the risk of GI injury increases independently and sometimes synergistically. In high-risk patients who require NSAID administration, examination of *H. pylori* infection is recommended followed by eradication therapy to prevent peptic ulcer in patients with confirmed *H. pylori* infection. A similar strategy is recommended for initial NSAID (excluding aspirin) users and long-term aspirin users with a history of peptic ulcer complications. In countries where the incidence of *H. pylori* infection is high, such as Korea, prevention and treatment strategies for NSAID users with *H. pylori* infection should differ from those in Western countries. Long-term multicenter studies that consider the current clinical situation are necessary in the future.

Keywords

Nonsteroidal anti-inflammatory drugs · *Helicobacter pylori* · Gastropathy · Peptic ulcer

31.1 Introduction

Helicobacter pylori (*H. pylori*) infection and nonsteroidal anti-inflammatory drug (NSAID) administration are known major risk factors for gastric and duodenal ulcers. With efforts to reduce the *H. pylori* infection rate and the widespread use of eradication therapy, the incidence of peptic ulcers induced by *H. pylori* infection has decreased. However, with an aging society worldwide, the prevalence rates of chronic musculoskeletal, cardiovascular, and cerebrovascular diseases have been increasing. Consequently, the incidence of complications induced by aspirin or NSAIDs, including peptic ulcers, is increasing.

NSAID-induced gastropathy develops at doses that inhibit prostaglandin production, increase mucosal permeability, and enhance gastric motility, resulting in neutrophil infiltration, free radical production, and eventually mucosal lesions [1]. Well-known risk factors for NSAID-induced gastropathy include increasing age and the presence of comorbidities, especially a history of peptic ulcer disease, liver cirrhosis, and cardiovascular disease. Long-term use of NSAIDs, *H. pylori* infection, smoking, chronic alcohol consumption, and concomitant use of other medications also increase the risk of NSAID-induced gastropathy [2].

NSAID-related ulcers may be improved by discontinuing drug administration. However, drug administration is difficult to discontinue in

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patients with chronic diseases, and in most cases, continuous treatment for ulcers is necessary. Moreover, in countries where the *H. pylori* infection rate is high, such as Korea, many NSAID-related ulcers are accompanied by *H. pylori* infection; notably, in the presence of both of these factors, the risk of gastrointestinal (GI) complications increases. NSAIDs and *H. pylori* infection are independent risk factors and may also have a synergistic effect on the development of peptic ulcers and ulcer bleeding [3]. According to a systematic review that included 10,146 patients in 21 studies, peptic ulcers were more common in NSAID users with *H. pylori* infection (odds ratio [OR] 1.81; 95% confidence interval [CI], 1.40–2.36) [4]. *H. pylori* infection and NSAID use increased the risk of ulcer bleeding by 1.79- and 4.85-fold, respectively, whereas the combination of the two factors increased the risk by 6.13-fold [5].

Some reports have indicated that the incidence of complications has decreased with the application of *H. pylori* eradication therapy, but other reports have indicated no significant difference in the presence of *H. pylori*. Although various studies have investigated the effect of *H. pylori* infection on the development of gastritis or ulcers in patients receiving NSAIDs, controversies remain. This chapter describes the gastric damage related to NSAID administration, pathophysiological mechanisms, and treatment of ulcers induced by NSAID administration and *H. pylori* infection.

31.2 NSAID-Induced Gastropathy

31.2.1 Mechanisms

The GI mucosa can be damaged by various mechanisms induced by NSAID administration [6–10] (Fig. 31.1). First, NSAIDs can directly act on the GI mucosa. When administered orally, NSAIDs bind to the hydrogen ions in the gastric juice and penetrate the cell membrane of the epithelial cells of the GI mucosa. This induces intracellular acidification and inhibits intracellular mitochondrial function, thereby inducing cell apoptosis. NSAIDs can also act in a systemic manner. The absorbed NSAIDs inhibit cyclooxygenase (COX) activity and reduce endogenous prostaglandin (PG) synthesis and thus inhibit the GI mucosal defensive mechanism induced by PG. A decrease in PG synthesis results in decreased mucus and bicarbonate secretion from the epithelial cells, mucosal blood flow, and proliferation, and resistance to epithelial cell injury. These consecutive events lead to damage to the GI mucosa. Moreover, with a relative increase in lipoxygenase (LOX) activity compared to inhibition of COX activity, leukotriene (LT) production increases. Increased LT production induces spasm in the vascular endothelial cells, leading to ischemia and reperfusion stress, which increases reactive oxygen species production, thus resulting in inflammation and tissue apoptosis that induce damage to the GI mucosa. It also increases the production of inflammatory mediators such as tumor necrosis factor (TNF), inducing damage to the GI mucosa. Of the above-mentioned mechanisms, the major pathophysiological mechanism of peptic ulcers induced by repetitive NSAID exposure is thought to be the systemic action of NSAIDs, which inhibits COX activity in the GI mucosa.

31.2.2 Nonselective and Selective NSAIDs

Nonselective NSAIDs (e.g., indomethacin, naproxen, diclofenac, piroxicam, or ibuprofen) inhibit both COX-1 and COX-2 expressions related to PG synthesis. As an essential constitutive enzyme expressed in normal cells, COX-1 is used to produce PG for normal GI mucosa defense. COX-2 is usually induced by inflammation-inducing cytokines, such as endotoxins, TNF-α, or interleukin (IL)-1β, in pathological conditions. Although aspirin is classified as a nonselective NSAID, it partially induces a COX-2-mediated response. Once aspirin is administered, the COX-2-mediated response that converts arachidonic acid to 15-hydroxyeicosatetraenoic acid (15-HETE) is induced, and 15-HETE is subsequently con-

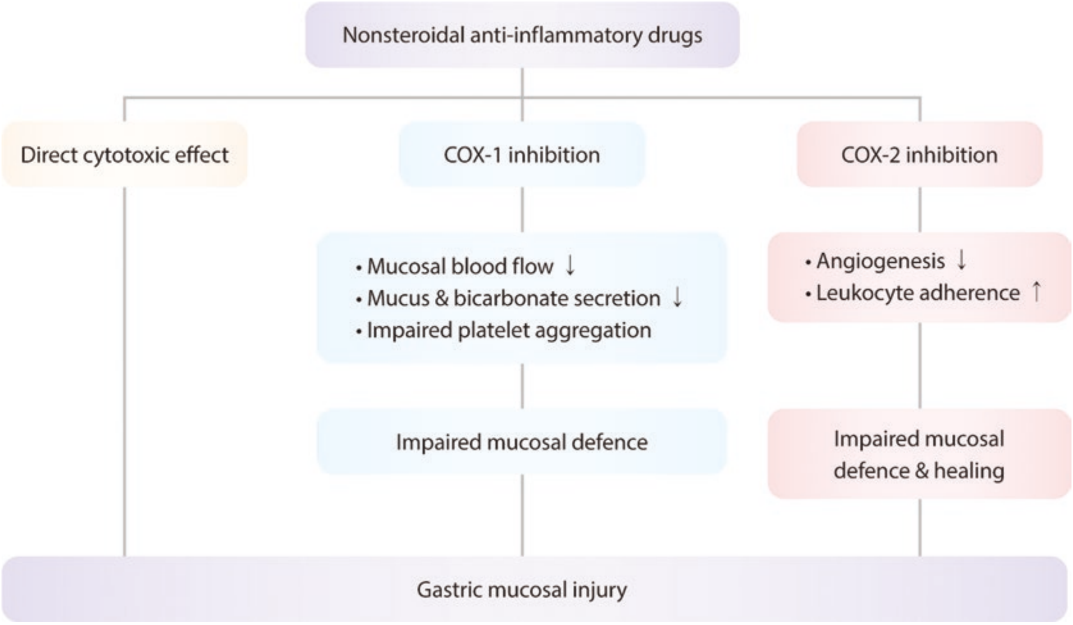
Nonsteroidal anti-inflammatory drugs
Direct cytotoxic effect
COX-1 inhibition
COX-2 inhibition
• Mucosal blood flow ↓
• Mucus & bicarbonate secretion ↓
• Impaired platelet aggregation
• Angiogenesis ↓
• Leukocyte adherence ↑
Impaired mucosal defence
Impaired mucosal defence & healing
Gastric mucosal injury

Fig. 31.1 Mechanisms of nonsteroidal anti-inflammatory drug (NSAID)-induced gastric mucosal injury

verted to 15-epi-lipoxin A4, minimizing gastric damage caused by 5-LOX production induced by aspirin administration. Accordingly, compared to other nonselective NSAIDs, aspirin acts as a stronger inhibitor of COX-1 than COX-2. With such a mechanism, the co-administration of low-dose aspirin and a selective COX-2 inhibitor would cause greater GI damage compared to the administration of aspirin alone [10, 11].

Selective COX-2 inhibitors (e.g., celecoxib or etodolac) can conserve the GI mucosal defense mechanism induced by PG via COX-1. However, it has been shown that compared with the placebo control group, the group that received COX-2 inhibitors showed greater damage to the GI mucosa. COX-2 expression is increased in peptic ulcers and GI inflammation to increase PG production for ulcer healing and to reduce inflammation. It is thought that administration of a selective COX-2 inhibitor damages the GI mucosa by blocking the protective effects of COX-2. In addition, a selective COX-2 inhibitor can also inhibit COX-1 expression at the clinically recommended dosage, thus indicating the possibility of GI mucosal damage even with its single administration [12, 13].

31.3 Association Between NSAID-Induced Gastropathy and *H. pylori* Infection

31.3.1 Pathophysiology

GI damage caused by NSAIDs or *H. pylori* occurs via different mechanisms, and the coexistence of these two factors independently increases the risk of GI damage [3, 4]. These two factors participate in the life cycle of GI epithelial cells. *H. pylori* infection induces a chronic hyperplastic state in the GI mucosa and promotes the apoptosis of epithelial cells. NSAIDs inhibit normal cell life cycles and cell growth and promote apoptosis of epithelial cells. Moreover, these two factors reduce mucosal viscosity and increase GI damage [14].

The incidence of ulcer occurrence in the *H. pylori* infection group was 4.03 times higher than that in the non-infection group, regardless of NSAID administration, and the incidence of ulcer occurrence in the NSAID administration group was 3.10 times higher than that in the non-NSAID administration group, regardless of *H. pylori* infection [15]. Furthermore, NSAID administration and *H. pylori* infection increased the risk of peptic ulcer bleeding by 4.8 and 1.8 times, respectively, and *H. pylori* infection in patients with NSAID administration showed a 61 times higher incidence rate of peptic ulcer and a six times higher risk of developing bleeding than patients without *H. pylori* infection [3]. In addition, the risk of uncomplicated peptic ulcer was 1.81 times higher in *H. pylori*-positive NSAID users than in *H. pylori*-negative NSAID users [4]. These findings support the fact that these two factors represent independent and synergistic risks for uncomplicated and bleeding peptic ulcers. Similar results have been observed in patients consuming aspirin. When aspirin was administered to patients with *H. pylori* infection, damage to the GI mucosa reached a maximum on day 3 of administration and continued until day 14. However, when aspirin was administered after *H. pylori* eradication therapy, the damage to the GI mucosa slowly decreased from day 3 [16].

However, another study reported that *H. pylori* infection might be helpful for healing peptic ulcers in patients who receive NSAID administration [17]. *H. pylori* infection induces excessive COX-2 mRNA expression related to growth factor, PG synthesis, and epithelial proliferation. This essentially results in increased PG production in the GI mucosa, which is helpful for ulcer healing and reduces GI complications due to NSAID administration. Considering this mechanism, when *H. pylori* eradication therapy is administered to patients receiving NSAIDs, ulcer healing may be delayed [15, 17].

31.3.2 Diagnosis of *H. pylori* Infection in NSAID Users

In Korea, to prevent peptic ulcers in high-risk patients who need NSAID treatment, tests for *H. pylori* infection are needed, and eradication therapy is recommended for confirmed *H. pylori* infection. Tests for *H. pylori* infection are also recommended when eradication therapy is considered effective in patients who have received an initial NSAID (excluding aspirin) treatment and in those receiving long-term aspirin administration with a history of peptic ulcer complications. However, tests are not recommended in patients receiving long-term NSAID (including aspirin) without any history of peptic ulcer complications and in patients receiving long-term NSAID (excluding aspirin) with a history of peptic ulcer complications [18, 19].

Meanwhile, in countries such as the United States and Canada, where the incidence rate of *H. pylori* infection is low, testing for *H. pylori* infection is recommended in all patients who have been receiving long-term NSAID administration regardless of risk factors [19, 20]. In countries such as Korea, where the incidence of *H. pylori* infection is high, testing for *H. pylori* infection is deemed necessary only when eradication therapy is expected to be effective. Hence, this approach is different from the guidelines recommended in Western countries.

31.3.3 Therapeutic Approach (Table 31.1)

31.3.3.1 *H. pylori* Infection in Initial NSAID Users

NSAIDs (Excluding Aspirin)

In a comparison between the *H. pylori* eradication therapy group and the non-eradication therapy group before 8-week naproxen administration in patients with *H. pylori* infection, *H. pylori* eradication therapy before NSAID administration reduced the occurrence of NSAID-related peptic ulcers [21]. In patients who were NSAID-naive, had a positive urea breath test, had dyspepsia or an ulcer history, and required long-term NSAID treatment, *H. pylori* eradication therapy before diclofenac slow-release administration for 6 months significantly reduced the risk of ulcers and complications [22]. Therefore, *H. pylori* eradication therapy reduces the incidence of peptic ulcers and complications in patients who have received an initial NSAID (excluding aspirin) administration.

Table 31.1 Evidence-based recommendation of *H. pylori* eradication in NSAID or aspirin users

Drug	Medication status	*H. pylori* eradication
NSAID	Initial user	Recommended
	Long-term user	
	Ulcer complication (−)	Not recommended
	Ulcer complication (+)	Not recommended (PPI is more effective)
Aspirin	Initial user	Not recommended
	Long-term user	
	Ulcer complication (−)	Not recommended
	Ulcer complication (+)	Recommended

Aspirin

Although *H. pylori* eradication therapy is recommended for some patients who have received initial aspirin administration, whether the treatment is necessary remains unclear because of the lack of definite evidence. In other words, the application of *H. pylori* eradication therapy is limited without the availability of systematized studies.

31.3.3.2 *H. pylori* Infection in Long-Term NSAID Users Without Peptic Ulcer Complications

NSAIDs (Excluding Aspirin)

In a randomized, double-blind, multicenter study of *H. pylori*-positive patients requiring NSAID therapy who had no past or current peptic ulcer, no difference was observed in the occurrence of peptic ulcers between the anti-*H. pylori* treatment, followed by proton-pump inhibitor (PPI) use and PPI treatment only [23]. Since there is no evidence showing that *H. pylori* eradication therapy accelerates ulcer healing in long-term NSAID (excluding aspirin) users without peptic ulcer complications, *H. pylori* eradication therapy is not recommended, and concomitant PPI administration is deemed necessary during the course of NSAID treatment.

Aspirin

The efficacy of *H. pylori* eradication therapy in long-term aspirin users without peptic ulcer complications has not been reported. Thus, whether *H. pylori* eradication therapy is required in long-term aspirin users without a history of peptic ulcer complications remains unclear.

31.3.3.3 *H. pylori* Infection in Long-Term NSAID Users with Peptic Ulcer Complications

NSAIDs (Excluding Aspirin)

When long-term naproxen users with a history of peptic ulcer complications randomly received PPI administration or 1 week of eradication therapy and were followed for 6 months, PPI was superior to *H. pylori* eradication therapy in preventing recurrent bleeding [24]. In other words, in long-term NSAID (excluding aspirin) users with a history of peptic ulcer complications, continuous PPI administration is more effective than *H. pylori* eradication therapy in inhibiting the development of peptic ulcer complications.

Aspirin

When long-term aspirin users with a history of bleeding peptic ulcer randomly received PPI administration or 1 week of eradication therapy and were followed for 6 months, *H. pylori* eradication therapy was equivalent to PPI treatment in preventing recurrent bleeding [24]. In another study, patients who had ulcer complications after using low-dose aspirin continuously for >1 month and who had *H. pylori* infection were randomly assigned to PPI treatment or placebo after *H. pylori* eradication therapy and were followed up for 12 months [25]. In this study, the rate of recurrence of ulcer complications was significantly lower with PPI treatment in addition to *H. pylori* eradication therapy. These findings indicated that *H. pylori* eradication therapy can reduce the recurrence of ulcer complications in long-term aspirin users with a history of ulcer complications.

31.3.3.4 *H. pylori* Infection in COX-2 Inhibitor Users

H. pylori infection often increases COX-2 mRNA expression, which is related to PG synthesis, epithelial cell proliferation, and growth factor expression. Some animal studies have shown that selective COX-2 inhibitors might aggravate damage to the GI mucosa in the presence of *H. pylori* infection [26, 27]. However, there are some limitations due to the lack of substantial studies.

31.3.3.5 Meta-analysis Results

A meta-analysis of five randomized trials with 939 patients receiving NSAIDs showed that 7.4% of patients developed an ulcer in the *H. pylori*-eradicated group compared with 13.3% in the control group (OR 0.43; 95% CI, 0.20–0.93) [28]. In the sub-analyses, the risk was reduced significantly in NSAID-naive patients (OR 0.26; 95% CI, 0.14–0.49) but not in previously NSAID-treated patients (OR 0.95; 95% CI, 0.53–1.72). When two studies ($n = 385$) comparing *H. pylori* eradication with PPI were included, the incidence of peptic ulcer disease was higher in the eradication group than in the non-eradication plus PPI group (OR 7.43; 95% CI, 1.27–43.64). Overall *H. pylori* eradication reduced the incidence of peptic ulcer in NSAID users; however, this benefit was noticed in NSAID-naive patients only. *H. pylori* eradication seems less effective than maintaining PPI treatment in preventing NSAID-associated ulcers.

Further, a recent meta-analysis including 10 cross-sectional studies and seven randomized controlled trials ($n = 5964$) showed that *H. pylori* infection increased the risk of gastroduodenal ulcers during low-dose aspirin therapy by approximately 70% (OR 1.68; 95% CI, 1.40–2.02) and in the absence of antisecretory drugs, the risk was almost double (OR 1.94; 95% CI, 1.54–2.46) [29]. These results may suggest the use of a test-and-treat approach to *H. pylori* infection in low-dose aspirin users, but at least 100 patients would need to be treated to prevent one bleeding event [30]. Therefore, a test-and-treat approach should be offered to patients at a higher risk of peptic ulcer and its complications.

31.3.4 Guidelines for Management of *H. pylori* Infection in NSAID Users

31.3.4.1 Korea

Clinical Guidelines for Drug-Related Peptic Ulcer, 2020 Revised Edition [31]

- We recommend that patients scheduled for long-term NSAID therapy undergo testing for, and treatment of, *H. pylori* infection in order to prevent peptic ulcer and its complications (recommendation, strong; evidence level, high).
- We recommend that patients who have a history of peptic ulcer receiving long-term low-dose aspirin therapy undergo treatment for *H. pylori* infection in order to prevent peptic ulcer and its complications (recommendation, strong; evidence level, low).

31.3.4.2 Overseas Countries

Management of *H. pylori* Infection: The Maastricht VI/Florence Consensus Report [32]

- The use of either aspirin or NSAIDs increases the risk of peptic ulcer disease and its complications in *H. pylori*-infected subjects (grading, A1; agreement, 100%).
- *H. pylori* testing and treatment are advisable for high-risk patients who are already on long-term aspirin. *H. pylori* testing and treatment are advisable for naive patients starting long-term NSAID therapy. Those at high risk may need additional PPI therapy (grading, A1; agreement, 100%).

Treatment of *H. pylori* Infection: ACG Clinical Guideline [33]

- In patients taking long-term, low-dose aspirin, testing for *H. pylori* infection could be considered to reduce the risk of ulcer bleeding. Those who test positive should be offered eradication therapy to reduce the risk of ulcer bleeding (recommendation, conditional; evidence level, moderate).

- Patients initiating chronic treatment with an NSAID should be tested for *H. pylori* infection. Those who test positive should be offered eradication therapy (recommendation, strong; evidence level, moderate).
- The benefit of testing and treating *H. pylori* in a patient already taking an NSAID remains unclear (recommendation, conditional; evidence level, low).

Management of *H. pylori* Infection: Italian Guideline [34]

- *H. pylori* should be searched for and eradicated in patients taking NSAIDs or acetylsalicylic acid (ASA) with a history of peptic ulcer. The eradication is more beneficial before starting NSAIDs or ASA therapy in preventing complicated and uncomplicated gastroduodenal peptic ulcers (recommendation, strong; evidence level, moderate).

Evidence-Based Clinical Practice Guidelines for Peptic Ulcer Disease 2020 in Japan [35]

- Eradication of *H. pylori* is recommended for the prevention of ulcers in NSAID-naive patients (recommendation, strong; agreement, 100%; evidence level, A).
- PPIs in addition to the eradication of *H. pylori* infection are recommended for the reduction in the incidence and prevalence rate of low-dose aspirin-related peptic ulcer bleeding (recommendation, strong; agreement, 100%; evidence level, B).
- H_2RAs in addition to the eradication of *H. pylori* infection are suggested for the reduction in the incidence and prevalence rate of low-dose aspirin-related peptic ulcer bleeding (recommendation, weak; agreement, 100%; evidence level, C).

31.4 Conclusions

With an aging society, the use of NSAIDs at their own discretion without physician prescription has increased. Thus, the incidence of NSAID-related peptic ulcer disease is continuously increasing. In addition, with the high incidence rate of *H. pylori* infection in Korea, the relationship between NSAIDs and *H. pylori* infection and their effects on GI injury is predicted to become an important issue in the treatment of peptic ulcer disease. Although most clinicians worldwide diagnose and treat *H. pylori* infection in patients with a history of peptic ulcer, regardless of NSAID administration, this issue remains controversial. Thus, long-term, multicenter studies that consider the current clinical situation are necessary in the future.

References

1. Takeuchi K. Pathogenesis of NSAID-induced gastric damage: importance of cyclooxygenase inhibition and gastric hypermotility. World J Gastroenterol. 2012;18:2147–60.
2. Hunt R, Lazebnik BL, Marakhouski YC, Manuc M, Gn R, Aye KS, et al. International consensus on guiding recommendations for management of patients with nonsteroidal antiinflammatory drugs induced gastropathy-ICON-G. Eur J Hepatogastroenterol. 2018;8:148–60.
3. Huang JQ, Sridhar S, Hunt RH. Role of *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. Lancet. 2002;359:14–22.
4. Papatheodoridis GV, Sougioultzis S, Archimandritis AJ. Effects of *Helicobacter pylori* and nonsteroidal anti-inflammatory drugs on peptic ulcer disease: a systematic review. Clin Gastroenterol Hepatol. 2006;4:130–42.
5. Harirforoosh S, Asghar W, Jamali F. Adverse effects of nonsteroidal antiinflammatory drugs: an update of gastrointestinal, cardiovascular and renal complications. J Pharm Pharm Sci. 2013;16:821–47.
6. Schlansky B, Hwang JH. Prevention of nonsteroidal anti-inflammatory drug-induced gastropathy. J Gastroenterol. 2009;44 (Suppl 19):44–52.
7. Sinha M, Gautam L, Shukla PK, Kaur P, Sharma S, Singh TP. Current perspectives in NSAID-induced gastropathy. Mediat Inflamm. 2013;2013:258209.
8. Wallace JL. NSAID gastropathy and enteropathy: distinct pathogenesis likely necessitates distinct prevention strategies. Br J Pharmacol. 2012;165:67–74.
9. Cryer B, Feldman M. Effects of very low dose daily, long-term aspirin therapy on gastric, duodenal, and rectal prostaglandin levels and on mucosal injury in healthy humans. Gastroenterology. 1999;117:17–25.
10. Iwamoto J, Saito Y, Honda A, Matsuzaki Y. Clinical features of gastroduodenal injury associated with long-term low-dose aspirin therapy. World J Gastroenterol. 2013;19:1673–82.

11. Fiorucci S, de Lima OM Jr, Mencarelli A, Palazzetti B, Distrutti E, McKnight W, et al. Cyclooxygenase-2-derived lipoxin A4 increases gastric resistance to aspirin-induced damage. Gastroenterology. 2002;123:1598–606.
12. Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, et al. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: a randomized controlled trial. Celecoxib long-term arthritis safety study. JAMA. 2000;284:1247–55.
13. Wallace JL. Pathogenesis of NSAID-induced gastroduodenal mucosal injury. Best Pract Res Clin Gastroenterol. 2001;15:691–703.
14. Wardi J, Shirin H. Non-steroidal anti-inflammatory drug-induced gastroduodenal toxicity and *Helicobacter pylori*. Isr Med Assoc J. 2003;5:195–7.
15. Papatheodoridis GV, Archimandritis AJ. Role of *Helicobacter pylori* eradication in aspirin or non-steroidal anti-inflammatory drug users. World J Gastroenterol. 2005;11:3811–6.
16. Konturek JW, Dembinski A, Konturek SJ, Stachura J, Domschke W. Infection of *Helicobacter pylori* in gastric adaptation to continued administration of aspirin in humans. Gastroenterology. 1998;114:245–55.
17. Halter F, Tarnawski AS, Schmassmann A, Peskar BM. Cyclooxygenase 2-implications on maintenance of gastric mucosal integrity and ulcer healing: controversial issues and perspectives. Gut. 2001;49:443–53.
18. Kim SG, Jung HK, Lee HL, Jang JY, Lee H, Kim CG, et al. Guidelines for the diagnosis and treatment of *Helicobacter pylori* infection in Korea, 2013 revised edition. J Gastroenterol Hepatol. 2014;29:1371–86.
19. Lee JH, Lee YC, Jeon SW, Kim JW, Lee SW, et al. [Guidelines of prevention and treatment for NSAID-related peptic ulcers]. Korean J Gastroenterol. 2009;54:309–17.
20. Graham DY, Chan FK. NSAIDs, risks, and gastroprotective strategies: current status and future. Gastroenterology. 2008;134:1240–6.
21. Chan FK, Sung JJ, Chung SC, To KF, Yung MY, Leung VK, et al. Randomised trial of eradication of *Helicobacter pylori* before non-steroidal anti-inflammatory drug therapy to prevent peptic ulcers. Lancet. 1997;350:975–9.
22. Chan FK, To KF, Wu JC, Yung MY, Leung WK, Kwok T, et al. Eradication of *Helicobacter pylori* and risk of peptic ulcers in patients starting long-term treatment with non-steroidal anti-inflammatory drugs: a randomised trial. Lancet. 2002;359:9–13.
23. Labenz J, Blum AL, Bolten WW, Dragosics B, Rosch W, Stolte M, et al. Primary prevention of diclofenac associated ulcers and dyspepsia by omeprazole or triple therapy in *Helicobacter pylori* positive patients: a randomised, double blind, placebo controlled, clinical trial. Gut. 2002;51:329–35.
24. Chan FK, Chung SC, Suen BY, Lee YT, Leung WK, Leung VK, et al. Preventing recurrent upper gastrointestinal bleeding in patients with *Helicobacter pylori* infection who are taking low-dose aspirin or naproxen. N Engl J Med. 2001;344:967–73.
25. Lai KC, Lam SK, Chu KM, Wong BC, Hui WM, Hu WH, et al. Lansoprazole for the prevention of recurrences of ulcer complications from long-term low-dose aspirin use. N Engl J Med. 2002;346:2033–8.
26. Futagami S, Hiratsuka T, Wada K, Tatsuguchi A, Tsukui T, Miyake K, et al. Inhibition of *Helicobacter pylori*-induced cyclo-oxygenase-2 aggravates NSAID-caused gastric damage in Mongolian gerbils. Aliment Pharmacol Ther. 2002;16:847–55.
27. Kim JM, Kim JS, Jung HC, Song IS, Kim CY. Upregulated cyclooxygenase-2 inhibits apoptosis of human gastric epithelial cells infected with *Helicobacter pylor*i. Dig Dis Sci. 2000;45:2436–43.
28. Vergara M, Catalan M, Gisbert JP, Calvet X. Meta-analysis: role of *Helicobacter pylori* eradication in the prevention of peptic ulcer in NSAID users. Aliment Pharmacol Ther. 2005;21:1411–8.
29. Sarri GL, Grigg SE, Yeomans ND. *Helicobacter pylor*i and low-dose aspirin ulcer risk: a meta-analysis. J Gastroenterol Hepatol. 2019;34:517–25.
30. Sostres C, Lanas A. Low dose aspirin, *H. pylori* infection, and the risk of upper gastrointestinal bleeding. Med J Aust. 2018;209:297–8.
31. Joo MK, Park CH, Kim JS, Park JM, Ahn JY, Lee BE, et al. Clinical guidelines for drug-related peptic ulcer, 2020 revised edition. Gut Liver. 2020;14:707–26.
32. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
33. Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG clinical guideline: treatment of *Helicobacter pylor*i infection. Am J Gastroenterol. 2017;112:212–39.
34. Romano M, Gravina AG, Eusebi LH, Pellegrino R, Palladino G, Frazzoni L, et al. Management of *Helicobacter pylor*i infection: guidelines of the Italian Society of Gastroenterology (SIGE) and the Italian Society of Digestive Endoscopy (SIED). Dig Liver Dis. 2022;54:1153–61.
35. Kamada T, Satoh K, Itoh T, Ito M, Iwamoto J, Okimoto T, et al. Evidence-based clinical practice guidelines for peptic ulcer disease 2020. J Gastroenterol. 2021;56:303–22.

Extraintestinal Manifestations of *H. pylori* Infection: *H. pylori*-Associated Iron Deficiency Anemia

32

Yon Ho Choe

Abstract

Adolescents are particularly susceptible to iron deficiency because of their high requirements for iron during the growth spurt and menstrual blood loss in girls. More iron intake and prompt absorption are needed at puberty. *Helicobacter pylori* (*H. pylori*) infection in the gastric mucosa might lead to iron deficiency anemia (IDA) because it seems to sequester iron and accordingly disturb iron absorption in adolescents whose iron supply is marginal, although the mechanisms by which *H. pylori* infection contributes to IDA remain unclear. When pubescent children or young adults are found to have IDA without evidence of gastrointestinal bleeding, and their anemia is refractory to iron supplementation and unexplained, *H. pylori* infection should be suspected, and eradication of the bacteria is necessary.

Keywords

Helicobacter pylori · Iron deficiency · Iron deficiency anemia

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32.1 Introduction

Iron deficiency is the most common cause of anemia. It is caused by low intake of iron, chronic blood loss, malabsorption, and hemolysis. Upper and lower endoscopies are recommended to find out the gastrointestinal (GI) causes of iron deficiency anemia (IDA). However, GI lesions contributing to anemia are identified in about 50% of patients who undergo upper and/or lower endoscopies [1]. Many reports relating *Helicobacter pylori* (*H. pylori*) infection to IDA have been described for the past two decades. Interestingly, the patients have no evidence of GI bleeding and their anemia is refractory to iron supplementation and subsided only after *H. pylori* eradication. Currently, according to the American College of Gastroenterology Guideline and the Maastricht IV/Florence Consensus Report, *H. pylori* eradication is recommended in otherwise unexplained IDA [2, 3].

32.2 Clinical Studies

In 1993, Dufour et al. [4] reported a 7-year-old case of *H. pylori*-associated chronic antral gastritis without evidence of hemorrhage or clinical symptoms other than sideropenic anemia, which subsided after *H. pylori* eradication. Since then, there have been similar reports and most of the patients were adolescents [5–16] (Table 32.1).

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Table 32.1 Studies concerning a relationship between *H. pylori* and iron deficiency anemia

Author	Year	Country	Type of study	Subjects	Cases (*n*)	Results	References
Dufour	1993	Italy	Case report	Adolescent	1	Resolution of IDA after *H. pylori* eradication	[4]
Marignani	1997	Italy	Case report	Adolescent	1	Resolution of IDA after *H. pylori* eradication	[5]
Carnicer	1997	Spain	Case report	Child	1	Resolution of IDA after *H. pylori* eradication	[6]
Barabino	1999	Italy	Case series	Children	4	Resolution of IDA after *H. pylori* eradication	[7]
Choe	1999	Korea	Randomized controlled trial	Adolescents	43	*H. pylori*(+) IDA patients after eradication had increased Hb compared with placebo	[8]
Choe	2000	Korea	Population survey	Adolescents	375	*H. pylori*-associated IDA as a risk factor for subnormal growth at puberty	[9]
Konno	2000	Japan	Case series	Adolescents	6	Resolution of IDA after *H. pylori* eradication	[10]
Ashorn	2001	Finland	Case series	Children	7	Resolution of IDA after *H. pylori* eradication	[11]
Choe	2001	Korea	Controlled trial in IDA/*H. pylori* seropositives	Adolescents	12 athletes, 10 controls	Significant increases in Hb, iron, and ferritin after *H. pylori* eradication c/w controls given iron therapy	[12]
Sugiyama	2002	Japan	Case series	Adult females	2	Resolution of IDA after *H. pylori* eradication	[13]
Choe	2003	Korea	Population survey	Adolescents	937	Prevalence of *H. pylori* infection with refractory IDA much higher than in the normal population	[14]
Kostaki	2003	Greece	Case series	Children	3	Resolution of IDA after *H. pylori* eradication	[15]
Hacihanefioglu	2004	Turkey	Case series	Adult females	14	Increase in Hb, iron, transferrin saturation, no change in ferritin	[16]
Mahalanabis	2005	India	Randomized controlled trial	Children	169	Resolution of IDA after *H. pylori* eradication	[18]
Gessner	2006	Alaska	Randomized controlled trial	Children	219	Difficulty in interpretation in endemic area	[19]
Sarker	2008	Bangladesh	Randomized controlled trial	Children	200	Difficulty in interpretation in endemic area	[20]
Fagan	2009	Alaska	Randomized controlled trial	Children	219	Resolution of IDA after *H. pylori* eradication	[21]
Duque	2010	Mexico	Randomized controlled trial	Children	69	Resolution of IDA after *H. pylori* eradication	[22]
Cardenas	2011	Texas	Randomized controlled trial	Children	110	Increase in serum ferritin after *H. pylori* eradication	[23]
Xia	2012	China	Randomized controlled trial	Adolescents	80	Resolution of IDA after *H. pylori* eradication	[24]
Hudak	2017	Israel	Meta-analysis		64 studies	Resolution of IDA after *H. pylori* eradication	[25]

IDA iron deficiency anemia

The author, as a pediatrician, also has experienced refractory IDA patients whose stool occult blood tests and endoscopic findings showed negative except for positive *H. pylori* infection. *H. pylori* eradication resulted in cure of IDA. Interestingly, a considerable number of patients with *H. pylori*-associated IDA were adolescent athletes. In 1997, the author conducted a double-blinded, placebo-controlled trial in pubescent children with IDA and coexisting *H. pylori* infection [8]. Of 43 IDA subjects, 22 *H. pylori*-positive patients were assigned to three groups. Group A was given oral iron and a 2-week course of eradication, group B received placebo for iron and eradication treatment, and group C was given oral iron and a 2-week course of placebo. Group A and B subjects, who received eradication therapy, showed a significant increase in hemoglobin level as compared with group C at 8 weeks after therapy ($p = 0.0086$). In an open trial conducted in the same center, 11 out of 21 subjects with refractory IDA were confirmed to have *H. pylori* infection without evidence of GI bleeding, and they were given oral iron supplementation for 3 months but failed. The levels of hemoglobin and serum ferritin showed a prominent increase after the eradication of *H. pylori* ($p = 0.0002$) [17]. Since 2005, large-scale randomized controlled trials have been performed and they revealed strong evidence linking *H. pylori* to the etiology of unexplained IDA [18–24]. A recent meta-analysis study also confirmed these findings [25] (Table 32.1).

32.3 Epidemiology

In 1998, a Danish population survey was performed on 2794 adults, whose serum ferritin, hemoglobin, and IgG antibodies against *H. pylori* were assessed. The study showed that serum ferritin levels are reduced in people with increased IgG antibodies to *H. pylori*, suggesting that *H. pylori* infection affects iron metabolism in humans [26]. In an Australian study, the investigators measured *H. pylori* IgG antibodies, plasma ferritin, and iron intake in 160 women, which revealed that the ferritin concentration of infected women was significantly lower than for noninfected women despite the similar daily iron intakes [27]. A cross-sectional national health survey among 1806 healthy people in Germany showed that *H. pylori* infection was associated with a 17.0% decrease in the serum ferritin concentration. They also reported that the association between *H. pylori* infection and serum ferritin levels did not vary by gender, age, and iron intake, and it was similar for CagA-positive and CagA-negative *H. pylori* infections [28]. In 2000, the author conducted a population survey for 375 healthy children aged 10–15 years, which showed that the prevalence rate of *H. pylori* infection was 15.5% in children without IDA and 31.3% in those affected ($p = 0.022$) [9]. In another study for pubescent athletes, prevalence rates of IDA, *H. pylori* infection, and *H. pylori*-associated IDA in female athletes were higher than in the control group. The relative risk of IDA was 2.9 (95% confidence interval [CI], 1.5–5.6) for those with *H. pylori* infection [12]. In a large-scale seroprevalence study in 937 pubescent children, the *H. pylori*-positive rates in anemia, hypoferritinemia, and iron deficiency group were 34.2%, 29.5%, and 35.3%, respectively, compared to 19.6% in the non-anemia group ($p = 0.003$), 19.2% in the non-hypoferritinemia group ($p = 0.005$), and 19.4% in the non-iron deficiency group ($p = 0.001$) [14]. The *H. pylori*-positive rate in the IDA group was 44.8% in comparison with 20.0% in the non-IDA group ($p = 0.001$). The associations between iron status and *H. pylori* were largely restricted to girls rather than boys [14]. In another Korean study, the *H. pylori* seropositivity in 753 schoolchildren aged 6–12 years was measured, and serum ferritin levels were significantly lower in *H. pylori*-seropositive children than in *H. pylori*-seronegative controls ($p < 0.001$) [29].

Adolescents are susceptible to iron deficiency because of their high requirement for iron during the growth spurt at puberty, low dietary intake, and menstrual blood loss in girls. Sports anemia, reported for the first time in 1970, is multifactorial: dilutional pseudoanemia, intravascular hemolysis, starvation anemia, and IDA [30–33]. The most common type of true anemia that affects athletes is IDA. The frequent sources of iron deficiency are blood loss from the GI tract and menstruation, poor dietary intake, and iron loss through profuse sweating [34, 35]. The major risk factor for *H. pylori* infection is the socioeconomic status of the family during childhood, as reflected in the number of persons in a household and sharing a bed, absence of a fixed hot water supply, and poor sanitation [36–38]. In Korea, most adolescent athletes live together in training camps for a considerable period per year, usually between middle school and college. Therefore, these athletes are susceptible to *H. pylori* infection because of environmental factors. It is consistent with the data from a Korean seroprevalence study performed on 440 students from a regular high school and 220 athletes from a physical education high school; the prevalence of *H. pylori* infection in athletes (43.2%) was higher than that in the controls (22.7%; $p = 0.005$) [39]. Taken together, *H. pylori* infection seems to act as "iron stressor," especially in adolescent girls who are vulnerable to iron deficiency, rapidly growing children at puberty, and athletes who are at risk for sports anemia.

However, a couple of studies have reported that there is no association between *H. pylori* and IDA. In a study conducted in 1060 adults in New Zealand, there were no significant differences in serum ferritin in either males or females between *H. pylori*-seropositive and *H. pylori*-seronegative subjects [40]. A Korean study of 693 children aged 9–12 years showed no significant difference in the seroprevalence of *H. pylori* infection between the IDA group and the non-anemic controls [41]. However, these two reports have the limitation that adolescents who are vulnerable to iron deficiency are not included in the subjects.

In conclusion, the literature reveals that *H. pylori*-associated IDA develops mostly at puberty, when the high requirement for iron is necessary [42–44].

32.4 Mechanisms

32.4.1 Chronic Gastrointestinal Blood Loss Induced by *H. pylori* Infection

In 1991, a 15-year-old girl was reported to have syncope, leading to the diagnosis of *H. pylori*-positive chronic active hemorrhagic gastritis. Her hemoglobin values returned to normal, and the symptoms disappeared after eradication [45]. According to a study to confirm prevalent iron deficiency among Yupik Eskimos living in Alaska, occult GI bleeding appeared to be pervasive in the Yupik population and likely underlay the prevalent iron deficiency. They concluded that an atypical hemorrhagic gastritis associated with *H. pylori* infection was present almost universally and might represent the bleeding source [46].

However, most published reports have found no evidence of bleeding lesions at the time of endoscopy in patients with *H. pylori*-associated IDA.

32.4.2 The Effect of Chronic *H. pylori* Gastritis on Gastric Acid Secretion and Iron Absorption

Dietary iron is available as heme iron (meat), which is readily absorbed, or nonheme iron (vegetable, grain), in which bioavailability is dependent on a variety of factors [42]. Nonheme iron accounts for 80% of dietary iron in industrialized countries. Crucial to the effective solubility and absorption of nonheme iron is hydrochloric acid in acid secretions. Reduction of the ferric to ferrous form is dependent upon the pH of the gastric juice, and reduction to the ferrous form facilitates membrane transport [47]. Annibale et al. [48] reported a high frequency of chronic gastritis in the gastric body and antrum in patients with refractory IDA and *H. pylori* infection. They showed that 80% of their patients had chronic pangastritis. In patients with *H. pylori*-associated corpus gastritis, an inverse relationship was reported between the severity of gastritis and acid secretion, concluding that gastric function can recover to normal or almost normal after cure of *H. pylori* infection [49]. Another study showed that the number of parietal cells in the secretory phase was significantly lower in *H. pylori*-infected patients than in those without *H. pylori* infection, accounting for the higher intragastric pH in patients with *H. pylori*-associated gastritis [50].

An important promotor of iron absorption is ascorbic acid, appearing to act either by promoting reduction to the ferrous form or by forming an absorbable molecular complex with ferric iron, which is insoluble at pH values above 5 [51]. Normally, gastric acid appears to facilitate the chelation in the stomach of ferric salts with ascorbic acid and other substances. Since these chelates then remain soluble in the more alkaline duodenum and jejunum, iron absorption is enhanced [52, 53]. Ascorbic acid is secreted into the stomach at a high concentration, which would support the idea of active secretion. Chronic gastritis seems to interfere with this secretion and reduces the ascorbic acid concentration in the gastric juice [54]. It has been reported that the reduction in gastric ascorbic acid concentration is related to gastric juice pH, the severity and extent of gastritis, and the presence of *H. pylori* [55]. In an Italian study, the vast majority of patients with unexplained IDA and *H. pylori* gastritis were characterized by gastric body involvement, suggesting a plausible explanation for the negative effect of *H. pylori* gastritis on physiological alimentary iron absorption [56].

Annibale et al. [57] evaluated gastric juice pH and gastric juice and plasma ascorbic acid in patients with *H. pylori* infection and unexplained IDA, compared with controls with IDA and a healthy stomach or with controls with *H. pylori* infection and no IDA. They performed the upper and lower endoscopy in patients with unexplained IDA to exclude the possible causes of IDA. Forty-three patients were divided into two groups according to whether they had *H. pylori* gastritis (n = 30) or normal gastric histology ($n = 13$). In addition, 11 non-anemic patients with *H. pylori* gastritis were included as another control group. They found that mean intragastric pH concentrations were significantly higher in the patients with *H. pylori*-associated IDA compared with each control group. Ascorbic acid concentrations in gastric juice were significantly lower in *H. pylori*-associated IDA patients compared with IDA patients without *H. pylori* infection and non-anemic patients with *H. pylori* infection. They also reported that 94% of patients with IDA had pangastritis, whereas most of those without IDA had antral gastritis.

Assuming that *H. pylori* infection affects gastric acid secretion and ascorbic acid concentrations in gastric juice, we can suggest that those parameters be reversible after *H. pylori* eradication. However, only a small number of patients received *H. pylori* eradication therapy in the study, and there were no differences in gastric pH and ascorbic concentrations in patients with chronic atrophic corpus gastritis.

32.4.3 Increased Iron Consumption by *H. pylori*

Iron is an essential element for almost all living organisms, as it is a cofactor required for the activity of many enzymes [58]. Pathogenic bacteria need to develop specific iron uptake systems in order to thrive in the host environment [51]. In *H. pylori*, not only iron uptake but also iron storage is regulated by the ferric uptake regulator (fur), which controls iron transport in bacteria [58]. In iron-replete environments, *H. pylori* expresses the Pfr protein, and the FeoB protein is expressed in iron-restricted conditions.

The Pfr, 19.6 kDa bacterioferritin of *H. pylori*, is known to have a major role in storing iron that the bacterium sequestered, and avoiding toxicity to the cells, under conditions of constant or intermittent iron excess [59]. The author performed sequence comparisons for the *pfr* regions between 16 IDA-positive and 10 IDA-negative groups in *H. pylori* gastritis patients, which showed that the mutation in the *pfr* gene did not relate to the clinical phenotype, IDA [60].

FeoB-mediated Fe^{2+} uptake is known to be a major pathway for *H. pylori* iron acquisition.

FeoB mutants were unable to colonize the gastric mucosa of mice, indicating that FeoB makes an important contribution to iron acquisition by *H. pylori* in the low-pH, low-O_2 environment of the stomach [61].

The author categorized 14 *H. pylori* gastritis patients into subgroups based on the presence or absence of IDA. Eight patients were diagnosed as having IDA; the other six showed normal hematological findings. According to the sequence analysis of the complete coding region of *feoB* gene, the four polymorphisms of the *feoB* gene appeared to be related to the clinical phenotype of IDA, but the relation was unclear because of the small number of strains studied [62].

Studies regarding the impact of strain differences in iron regulatory genes on IDA are still controversial.

32.4.4 Iron Sequestration in Gastric Mucosa

Is it possible for iron that reached the stomach to be sequestered in some space without absorption by *H. pylori* infection? In 1999, Barabino et al. [7] reported that the ^{59}Fe red blood cell use decreased in all their case series, which might suggest that iron was diverted from the bone marrow to some extramedullary focus, possibly the *H. pylori* gastric infection. It was suggested that the iron acquisition system of *H. pylori* by the human lactoferrin receptor system might play a major role in the virulence of *H. pylori* infection, since lactoferrin was found in significant amounts in human stomach resections with gastritis [63]. In that study, investigators were not able to detect any siderophore production in the culture supernatants of *H. pylori* strains under iron-limited conditions. *H. pylori* can, however, use human lactoferrin and heme as an iron source [63]. The lack of siderophore production and the need for heme as a growth factor, which may increase significantly the availability of heme, suggest the existence of a specific heme uptake system in *H. pylori* [64]. Their electrophoretic analysis of outer membrane preparations from *H. pylori* cultured under conditions of iron restriction revealed a couple of proteins to be present at elevated levels, called iron-repressible outer membrane proteins. Dhaenens et al. [65] identified a 70 kDa lactoferrin-binding protein (Lbp) from the outer membrane proteins of *H. pylori*. This Lbp was only present when *H. pylori* was cultured in an iron-restricted medium, suggesting its role in iron uptake.

It has been known that lactoferrin concentration is increased in the biopsy specimens of patients with *H. pylori* gastritis and that the levels of lactoferrin correlate significantly with the degree of inflammation of the gastric mucosa [66]. Lactoferrin is an iron-binding glycoprotein that is found in various body fluids such as milk, lacrimal secretions, saliva, and urine [67]. It also has been found in the cardiac and pyloric glands and neutrophils within surface epithelium [68]. Lactoferrin binds free iron with great affinity limiting the amount of ions available for microorganism's metabolism. Therefore, its role in the host defense mechanisms consists of bacteriostatic and bactericidal effects. It is also involved in the modulation of the immune system and recent studies show that lactoferrin directly modulates both the production and function of neutrophils and monocytes [69].

However, *H. pylori* does not seem to be eradicated by lactoferrin, even though lactoferrin concentration is increased in gastric mucosa with *H. pylori* infection. Rather, it is assumed that *H. pylori* absorbs the iron from lactoferrin via a specific Lbp expressed by *H. pylori* [51]. Husson et al. [63] reported that human lactoferrin supported the full growth of the bacteria in media lacking other iron sources, but neither human transferrin and bovine lactoferrin nor hen ovotransferrin served as a source for iron.

Based on the studies described above, the author investigated the relationship between lactoferrin and *H. pylori* infection coexistent with IDA by determining the lactoferrin levels in gastric biopsy specimens, according to the presence or absence of iron deficiency anemia [70]. One hundred and one adolescents who underwent gastroduodenoscopy were divided into four groups: controls without *H. pylori* infection (NL; $n = 43$), patients with *H. pylori* infection (HP; $n = 26$), patients with IDA (IDA; *n* = 6), and patients with *H. pylori* gastritis and coexisting IDA (HPIDA; *n* = 26). The mucosal level of lactoferrin was highest in HPIDA, followed by HP, NL, and IDA. When mucosal lactoferrin and blood hemoglobin levels were compared before and after *H. pylori* eradication in 12 HPIDA patients who underwent follow-up endoscopy, lactoferrin levels decreased and hemoglobin levels increased significantly after eradication. These findings support the hypothesis that *H. pylori* gastritis could act as a sequestrating focus for iron [7]. Moreover, this hypothesis was also proved to be true by immunohistochemical staining. When the gastric mucosa is infected with *H. pylori*, lactoferrin secretion increases in the glands and neutrophils. Interestingly, the mucosal lactoferrin level in the patient with only IDA was the lowest, while it was the highest when the patient was affected by both *H. pylori* infection and IDA. The former result seems to reflect that the whole body is short of iron and iron-containing substances such as lactoferrin, and the latter seems to reveal that iron is sequestered in the gastric mucosa by *H. pylori* infection even though the host is suffering from iron deficiency.

Barabino speculated that *H. pylori*-infected antrum could act as a sequestering focus for iron: *H. pylori* infection enhances gastric lactoferrin. The iron bound to lactoferrin is in turn picked up by the bacterium, by means of its outer membrane receptors, for its own growth. As *H. pylori* turnover is very rapid, the bacterial iron stores are rapidly lost in the stools, together with the dead bacteria. This mechanism could explain why an iron supply is no longer available for hemopoiesis, which only enhances *H. pylori* proliferation [71].

32.4.5 Higher Hepcidin Levels in *H. pylori*-Infected Patients with Iron Deficiency Anemia

Hepcidin controls the dietary absorption, storage, and tissue distribution of iron by decreasing the iron transfer into the blood from the duodenum, from macrophages involved in recycling senescent erythrocytes, and from iron-storing hepatocytes [72]. It is increased by an elevated plasma iron concentration and inflammation. A case-control study showed that *H. pylori*-infected children with IDA had significantly higher serum hepcidin levels in comparison with noninfected children with IDA and healthy controls [73]. Hepcidin increased significantly in noninfected children with IDA after 3 months of oral iron therapy. On the other hand, *H. pylori*-infected children showed a nonsignificant change in hepcidin levels after oral iron therapy, suggesting that *H. pylori* infection upregulates serum hepcidin levels and is associated with diminished response to oral iron therapy in children with IDA [73]. A recent clinical trial revealed that hepcidin level decreased after successful *H. pylori* eradication with improvement in IDA [74].

32.5 Conclusions

Adolescents are particularly susceptible to iron deficiency because of their high requirements for iron during the growth spurt and menstrual blood loss in girls. Although more iron intake and prompt absorption are needed at puberty, *H. pylori* infection in the gastric mucosa might lead to IDA because it sequesters iron and accordingly disturbs iron absorption in adolescents whose iron supply is marginal. It was speculated that even a small negative balance in iron storage at puberty could contribute to IDA because pubescent children are at risk of iron deficiency [70]. This suggestion has been typically demonstrated in a study conducted in adolescent female athlete group [12] (Fig. 32.1). The above speculation gives us a plausible explanation for why only a small proportion of the population develops IDA in spite of worldwide *H. pylori* infection. A similar finding has been reported even in an Italian adult study, in which the majority of patients with *H. pylori*-associated IDA were premenopausal women who are vulnerable to iron loss [75].

The biological mechanisms for *H. pylori*-associated IDA should be assessed in the aspects of two sides (Fig. 32.2). One is a bacterial aspect, and the other, host. In the aspect of bacteria, the

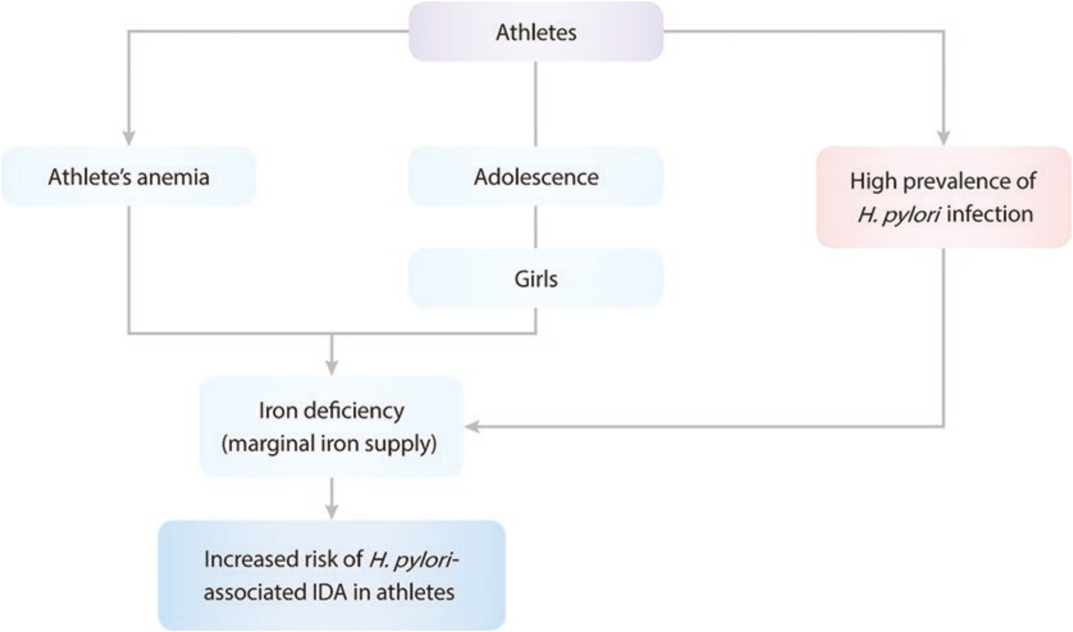
Athletes
Athlete's anemia
Adolescence
Girls
High prevalence of *H. pylori* infection
Iron deficiency (marginal iron supply)
Increased risk of *H. pylori*-associated IDA in athletes

Fig. 32.1 Flowchart of increased risk of *H. pylori*-associated IDA (iron deficiency anemia) in adolescent female athletes

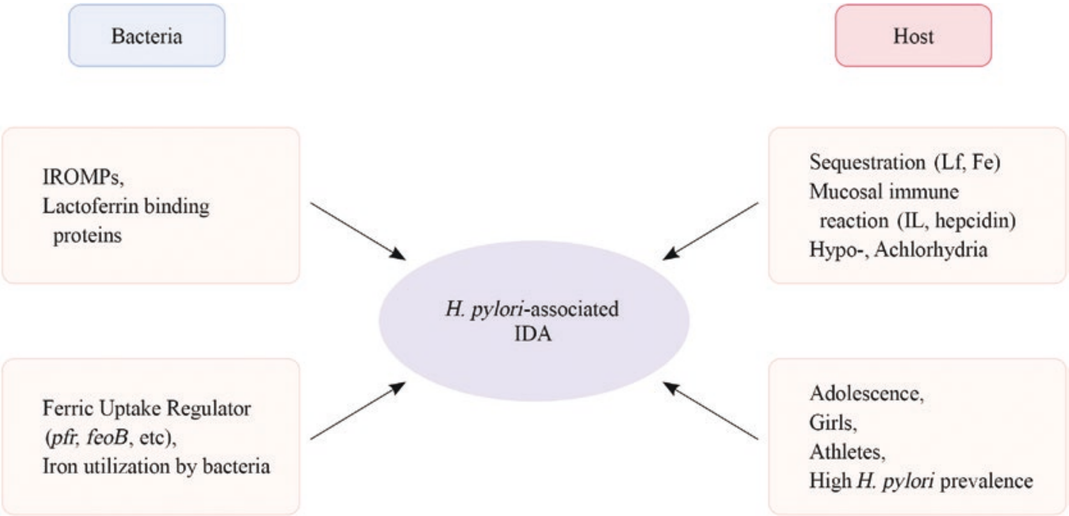
Bacteria
Host
IROMPs,
Lactoferrin binding proteins
Sequestration (Lf, Fe)
Mucosal immune reaction (IL, hepcidin)
Hypo-, Achlorhydria
H. pylori-associated IDA
Ferric Uptake Regulator (*pfr*, *feoB*, etc),
Iron utilization by bacteria
Adolescence,
Girls,
Athletes,
High *H. pylori* prevalence

Fig. 32.2 Possible mechanisms by which *H. pylori* infection contributes to iron deficiency anemia (IDA). *IROMPs* iron-repressible outer membrane proteins, *Lf* lactoferrin, *IL* interleukin

iron is overconsumed or the iron uptake system in the human body is altered by *H. pylori*. Another explanation is possible that there exist *H. pylori* strain differences contributing to IDA. On the contrary, host reactions to *H. pylori* infection causing IDA include the change in gastric acid secretion, iron sequestration, and accompanying mucosal immune reactions. To date, there has been no clear report that verified the decisive mechanisms by which *H. pylori* infection might lead to IDA. It appears that several pathways are involved separately or in combination [42].

When pubescent children or young adults are found to have IDA, and their anemia is refractory to iron supplementation and unexplained, *H. pylori* infection should be suspected and eradication of the bacteria is necessary. *H. pylori*-associated IDA can be cured by bacterial eradication.

References

1. McIntyre AS, Long RG. Prospective survey of investigations in outpatients referred with iron deficiency anaemia. Gut. 1993;34:1102–7.
2. Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG clinical guideline: treatment of *Helicobacter pylori* infection. Am J Gastroenterol. 2017;112:212–38.
3. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of *Helicobacter pylori* infection-the Maastricht IV/ Florence consensus report. Gut. 2012;61:646–64.
4. Dufour C, Brisigotti M, Fabretti G, Luxardo P, Mori PG, Barabino A. *Helicobacter pylori* gastric infection and sideropenic refractory anemia. J Pediatr Gastroenterol Nutr. 1993;17:225–7.
5. Marignani M, Angeletti S, Bordi C, Malagnino F, Mancino C, Delle Fave G, et al. Reversal of long-standing iron deficiency anaemia after eradication of *Helicobacter pylori* infection. Scand J Gastroenterol. 1997;32:617–22.
6. Carnicer J, Badía R, Argemí J. *Helicobacter pylori* gastritis and sideropenic refractory anemia. J Pediatr Gastroenterol Nutr. 1997;25:441.
7. Barabino A, Dufour C, Marino CE, Claudiani F, De Alessandri A. Unexplained refractory iron-deficiency anemia associated with *Helicobacter pylori* gastric infection in children: further clinical evidence. J Pediatr Gastroenterol Nutr. 1999;28:116–9.
8. Choe YH, Kim SK, Son BK, Lee DH, Hong YC, Pai SH. Randomized placebo-controlled trial of *Helicobacter pylori* eradication for iron-deficiency anemia in preadolescent children and adolescents. Helicobacter. 1999;4:135–9.
9. Choe YH, Kim SK, Hong YC. *Helicobacter pylori* infection with iron deficiency anaemia and subnormal growth at puberty. Arch Dis Child. 2000;82:136–40.
10. Konno M, Muraoka S, Takahashi M, Imai T. Iron-deficiency anemia associated with *Helicobacter pylori* gastritis. J Pediatr Gastroenterol Nutr. 2000;31:52–6.
11. Ashorn M, Ruuska T, Mäkipernaa A. *Helicobacter pylori* and iron deficiency anaemia in children. Scand J Gastroenterol. 2001;36:701–5.
12. Choe YH, Kwon YS, Jung MK, Kang SK, Hwang TS, Hong YC. *Helicobacter pylori*-associated iron-deficiency anemia in adolescent female athletes. J Pediatr. 2001;139:100–4.
13. Sugiyama T, Tsuchida M, Yokota K, Shimodan M, Asaka M. Improvement of long-standing iron-deficiency anemia in adults after eradication of *Helicobacter pylori* infection. Intern Med. 2002;41:491–4.
14. Choe YH, Kim SK, Hong YC. The relationship between *Helicobacter pylori* infection and iron deficiency: seroprevalence study in 937 pubescent children. Arch Dis Child. 2003;88:178.
15. Kostaki M, Fessatou S, Karpathios T. Refractory iron-deficiency anaemia due to silent *Helicobacter pylori* gastritis in children. Eur J Pediatr. 2003;162:177–9.
16. Hacihanefioglu A, Edebali F, Celebi A, Karakaya T, Senturk O, Hulagu S. Improvement of complete blood count in patients with iron deficiency anemia and *Helicobacter pylori* infection after the eradication of *Helicobacter pylori*. Hepato-Gastroenterology. 2004;51:313–5.
17. Choe YH, Lee JE, Kim SK. Effect of *Helicobacter pylori* eradication on sideropenic refractory anaemia in adolescent girls with *Helicobacter pylori* infection. Acta Paediatr. 2000;89:154–7.
18. Mahalanabis D, Isram MA, Shaikh S, Chakrabarty M, Kurpad AV, Mukherjee S, et al. Haematological response to iron supplementation is reduced in children with asymptomatic *Helicobacter pylori* infection. Br J Nutr. 2005;94:969–75.
19. Gessner BD, Baggett HC, Muth PT, Dunaway E, Gold BD, Feng Z, et al. A controlled, household-randomized, open-label trial of the effect that treatment of *Helicobacter pylori* infection has on iron deficiency in children in rural Alaska. J Infect Dis. 2006;193:537–46.
20. Sarker SA, Mahmud H, Davidsson L, Alam NH, Ahmed T, Alam N, et al. Causal relationship of *Helicobacter pylori* with iron-deficiency anemia or failure of iron supplementation in children. Gastroenterology. 2008;135:1534–42.
21. Fagan RP, Dunaway CE, Bruden DL, Parkinson AJ, Gessner BD. Controlled, household-randomized, open-label trial of the effect of treatment of *Helicobacter pylori* infection has on iron deficiency among children in rural Alaska: results at 40 months. J Infect Dis. 2009;199:652–60.
22. Duque X, Moran S, Mera R, Medina M, Martinez H, Mendoza ME, et al. Effect of eradication of *Helicobacter pylori* and iron supplementation on the iron status of children with iron deficiency. Arch Med Res. 2010;41:38–45.
23. Cardenas VM, Prieto-Jimenez CA, Mulla ZD, Rivera JO, Dominguez DC, Graham DY, et al. *Helicobacter pylori* eradication and change in markers of iron stores among non-iron-deficient children in El Paso, Texas: an etiologic intervention study. J Pediatr Gastroenterol Nutr. 2011;52:326–32.
24. Xia W, Zhang X, Wang J, Sun C, Wu L. Survey of anaemia and *Helicobacter pylori* infection in adolescent girls in Suihua, China and enhancement of iron

intervention effects by *Helicobacter pylori* eradication. Br J Nutr. 2012;108:357–62.

25. Hudak L, Jaraisy A, Haj S, Muhsen K. An updated systematic review and meta-analysis on the association between *Helicobacter pylori* infection and iron deficiency anemia. Helicobacter. 2017;22:e12330.
26. Milman N, Rosenstock S, Andersen L, Jørgensen T, Bonnevie O. Serum ferritin, hemoglobin, and *Helicobacter pylori* infection: a seroepidemiologic survey comprising 2794 Danish adults. Gastroenterology. 1998;115:268–74.
27. Peach HG, Bath NE, Farish SJ. *Helicobacter pylori* infection: an added stressor on iron status of women in the community. Med J Aust. 1998;169:188–90.
28. Berg G, Bode G, Blettner M, Boeing H, Brenner H. *Helicobacter pylori* infection and serum ferritin: a population-based study among 1806 adults in Germany. Am J Gastroenterol. 2001;96:1014–8.
29. Seo JK, Ko JS, Choi KD. Serum ferritin and *Helicobacter pylori* infection in children: a seroepidemiologic study in Korea. J Gastroenterol Hepatol. 2002;17:754–7.
30. Yoshimura G. Anemia during physical training (sports anemia). Nutr Rev. 1970;28:251–3.
31. Clement DB, Sawchuk LL. Iron status and sports performance. Sports Med. 1984;1:65–74.
32. Eichner ER. Sports anemia, iron supplements, and blood doping. Med Sci Sports Exerc. 1992;24(Suppl 9):S315–8.
33. Newhouse IJ, Clement DB. Iron status in athletes: an update. Sports Med. 1988;5:337–52.
34. Nichols AW. Nonorthopedic problems in the aquatic athlete. Clin Sports Med. 1999;18:395–411. viii
35. Escanero JF, Villanueva J, Rojo A, Herrera A, del Diego C, Guerra M. Iron stores in professional athletes throughout the sports season. Physiol Behav. 1997;62:811–4.
36. Mendall MA, Goggin PM, Molineaux N, Levy J, Toosy T, Strachan D, et al. Childhood living conditions and *Helicobacter pylori* seropositivity in adult life. Lancet. 1992;339:896–7.
37. Malaty HM, Graham DY. Effect of childhood socioeconomic status on the current prevalence of *Helicobacter pylori* infection. Gut. 1994;35:742–5.
38. Webb PM, Knight T, Greaves S, Wilson A, Newell DG, Elder J, et al. Relation between infection with *Helicobacter pylori* and living conditions in childhood: evidence for person-to-person transmission in early life. BMJ. 1994;308:750–3.
39. Choe YH, Hwang TS, Hong YC. Higher seroprevalence of *Helicobacter pylori* infection in Korean adolescent athletes compared to age- and sex-matched non-athletes. J Gastroenterol Hepatol. 2002;17:131–4.
40. Collett JA, Burt MJ, Frampton CM, Yeo KH, Chapman TM, Buttimore RC, et al. Seroprevalence of *Helicobacter pylori* in the adult population of Christchurch: risk factors and relationship to dyspeptic symptoms and iron studies. N Z Med J. 1999;112:292–5.
41. Choi JW. Does *Helicobacter pylori* infection relate to iron deficiency anaemia in prepubescent children under 12 years of age? Acta Paediatr. 2003;92:970–2.
42. Pacifico L, Osborn JF, Tromba V, Romaggioli S, Bascetta S, Chiesa C. *Helicobacter pylori* infection and extragastric disorders in children: a critical update. World J Gastroenterol. 2014;20:1379–401.
43. Bagget HC, Parkinson AJ, Muth PT, Gold BD, Gessner BD. Endemic iron deficiency associated with *Helicobacter pylori* infection among school-aged children in Alaska. Pediatrics. 2006;117:e396–404.
44. Muhsen K, Barak M, Henig C, Alpert G, Ornoy A, Cohen D. Is the association between *Helicobacter pylori* infection and anemia age dependent? Helicobacter. 2010;15:467–72.
45. Blecker U, Renders F, Lanciers S, Vandenplas Y. Syncopes leading to the diagnosis of a *Helicobacter pylori* positive chronic active haemorrhagic gastritis. Eur J Pediatr. 1991;150:560–1.
46. Yip R, Limburg PJ, Ahlquist DA, Carpenter HA, O'Neill A, Kruse D, et al. Pervasive occult gastrointestinal bleeding in an Alaska native population with prevalent iron deficiency. Role of *Helicobacter pylori* gastritis. JAMA. 1997;277:1135–9.
47. Lombard M, Chua E, O'Toole P. Regulation of intestinal non-haem iron absorption. Gut. 1997;40:435–9.
48. Annibale B, Marignani M, Monarca B, Antonelli G, Marcheggiano A, Martino G, et al. Reversal of iron deficiency anemia after *Helicobacter pylori* eradication in patients with asymptomatic gastritis. Ann Intern Med. 1999;131:668–72.
49. Gutierrez O, Melo M, Segura AM, Angel A, Genta RM, Graham DY. Cure of *Helicobacter pylori* infection improves gastric acid secretion in patients with corpus gastritis. Scand J Gastroenterol. 1997;32:664–8.
50. Taguchi Y, Kaito M, Gabazza EC, Takaji S, Shibata T, Oka S, et al. *Helicobacter pylori* inhibits the secretory activity of gastric parietal cells in patients with chronic gastritis. An ultrastructural study. Scand J Gastroenterol. 1997;32:656–63.
51. Dubois S, Kearney DJ. Iron-deficiency anemia and *Helicobacter pylori* infection: a review of the evidence. Am J Gastroenterol. 2005;100:453–9.
52. Schade SG, Cohen RJ, Conrad ME. Effect of hydrochloric acid on iron absorption. N Engl J Med. 1968;279:672–4.
53. Kassarjian Z, Russell RM. Hypochlorhydria: a factor in nutrition. Annu Rev Nutr. 1989;9:271–85.
54. Schorah CJ, Sobala GM, Sanderson M, Collis N, Primrose JN. Gastric juice ascorbic acid: effects of disease and implications for gastric carcinogenesis. Am J Clin Nutr. 1991;53(Suppl 1):287–93S.
55. Zhang ZW, Patchett SE, Perrett D, Katelaris PH, Domizio P, Farthing MJ. The relation between gastric vitamin C concentrations, mucosal histology, and CagA seropositivity in the human stomach. Gut. 1998;43:322–6.

56. Capurso G, Lahner E, Marcheggiano A, Caruana P, Carnuccio A, Bordi C, et al. Involvement of the corporal mucosa and related changes in gastric acid secretion characterize patients with iron deficiency anaemia associated with *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2001;15:1753–61.
57. Annibale B, Capurso G, Lahner E, Passi S, Ricci R, Maggio F, et al. Concomitant alterations in intragastric pH and ascorbic acid concentration in patients with *Helicobacter pylori* gastritis and associated iron deficiency anaemia. Gut. 2003;52:496–501.
58. van Vliet AH, Stoof J, Vlasblom R, Wainwright SA, Hughes NJ, Kelly DJ, et al. The role of the ferric uptake regulator (fur) in regulation of *Helicobacter pylori* iron uptake. Helicobacter. 2002;7:237–44.
59. Doig P, Austin JW, Trust TJ. The *Helicobacter pylori* 19.6-kilodalton protein is an iron-containing protein resembling ferritin. J Bacteriol. 1993;175:557–60.
60. Choe YH, Hwang TS, Kim HJ, Shin SH, Song SU, Choi MS. A possible relation of the *Helicobacter pylori pfr* gene to iron deficiency anemia? Helicobacter. 2001;6:55–9.
61. Velayudhan J, Hughes NJ, McColm AA, Bagshaw J, Clayton CL, Andrews SC, et al. Iron acquisition and virulence in *Helicobacter pylori*: a major role for FeoB, a high-affinity ferrous iron transporter. Mol Microbiol. 2000;37:274–86.
62. Jeon BH, Oh YJ, Lee NG, Choe YH. Polymorphism of the *Helicobacter pylori feoB* gene in Korea: a possible relation with iron-deficiency anemia? Helicobacter. 2004;9:330–4.
63. Husson MO, Legrand D, Spik G, Leclerc H. Iron acquisition by *Helicobacter pylori*: importance of human lactoferrin. Infect Immun. 1993;61:2694–7.
64. Worst DJ, Otto BR, de Graaff J. Iron-repressible outer membrane proteins of *Helicobacter pylori* involved in heme uptake. Infect Immun. 1995;63:4161–5.
65. Dhaenens L, Szczebara F, Husson MO. Identification, characterization, and immunogenicity of the lactoferrin-binding protein from *Helicobacter pylori*. Infect Immun. 1997;65:514–8.
66. Nakao K, Imoto I, Ikemura N, Shibata T, Takaji S, Taguchi Y, et al. Relation of lactoferrin levels in gastric mucosa with *Helicobacter pylori* infection and with the degree of gastric inflammation. Am J Gastroenterol. 1997;92:1005–11.
67. Levay PF, Viljoen M. Lactoferrin: a general review. Haematologica. 1995;80:252–67.
68. Luqmani YA, Campbell TA, Bennett C, Coombes RC, Paterson IM. Express of lactoferrin in human stomach. Int J Cancer. 1991;49:684–7.
69. Cavestro GM, Ingegnoli AV, Aragona G, Iori V, Mantovani N, Altavilla N, et al. Lactoferrin: mechanism of action, clinical significance and therapeutic relevance. Acta Biomed. 2002;73:71–3.
70. Choe YH, Oh YJ, Lee NG, Imoto I, Adachi Y, Toyoda N, et al. Lactoferrin sequestration and its contribution to iron-deficiency anemia in *Helicobacter pylori*-infected gastric mucosa. J Gastroenterol Hepatol. 2003;18:980–5.
71. Barabino A. *Helicobacter pylori*-related iron deficiency anemia: a review. Helicobacter. 2002;7:71–5.
72. Ozkasap S, Yarali N, Isik P, Bay A, Kara A, Tunc B. The role of prohepcidin in anemia due to *Helicobacter pylori* infection. Pediatr Hematol Oncol. 2013;30:425–31.
73. Azab S, Esh A. Serum hepcidin levels in *Helicobacter pylori*-infected children with iron-deficiency anemia: a case–control study. Ann Hematol. 2013;92:1477–83.
74. Sapmaz F, Başyiğit S, Kalkan İH, Kısa Ü, Kavak EE, Güliter S. The impact of *Helicobacter pylori* eradication on serum hepcidin-25 level and iron parameters in patients with iron deficiency anemia. Wien Klin Wochenschr. 2016;128:335–40.
75. Ciacci C, Sabbatini F, Cavallaro R, Castiglione F, Di Bella S, Iovino P, et al. *Helicobacter pylori* impairs iron absorption in infected individuals. Dig Liver Dis. 2004;36:455–60.

Extraintestinal Manifestations of *H. pylori* Infection: Idiopathic Thrombocytopenic Purpura

33

Chan Gyoo Kim

Abstract

Epidemiologic studies, randomized controlled studies, and meta-analyses have reported an association between idiopathic thrombocytopenic purpura (ITP) and *Helicobacter pylori* (*H. pylori*) and the improvement of idiopathic thrombocytopenic purpura after *H. pylori* eradication. Because the underlying mechanism has not been determined, there are many hypotheses to explain the causal relationship between ITP and *H. pylori* infection. One hypothesis states that the antibodies associated with *H. pylori* infection, including the CagA protein, cross-react with glycoproteins on the surface of thrombocytes such as glycoprotein IIIa. *H. pylori* eradication is recommended for patients with idiopathic thrombocytopenic purpura and *H. pylori* infection in the guidelines about *H. pylori* in Europe, Japan, Asia-Pacific, and Korea.

Keywords

Helicobacter pylori · Eradication · Idiopathic thrombocytopenic purpura · Immune thrombocytopenic purpura

33.1 Introduction

In addition to idiopathic iron deficiency anemia, idiopathic thrombocytopenia (immune thrombocytopenia) is one of the hematologic diseases associated with *Helicobacter pylori* (*H. pylori*). For idiopathic thrombocytopenia, *H. pylori* eradication is recommended in guidelines for *H. pylori* in Europe (2022) [1], North America (USA 2017) [2], South America (Brazil 2018) [3], and Asia (Korea 2020, Japan 2016) [4, 5]. *H. pylori* eradication is also recommended in patients who have *H. pylori* infection in the American Society of Hematology 2019 guidelines for immune thrombocytopenia [6]. In this article, I review the association between *H. pylori* and idiopathic thrombocytopenia and the effect of *H. pylori* eradication on idiopathic thrombocytopenia.

33.2 Idiopathic Thrombocytopenic Purpura (Immune Thrombocytopenic Purpura)

Idiopathic thrombocytopenia occurs via an immune mechanism in which the level of thrombocytes decreases below the normal level (150,000/uL) due to the destruction of thrombocytes by an autoantibody or immune complex. It is referred to as idiopathic when the cause is not clearly determined and as secondary when the

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immune complex or autoantibody was produced as a result of drug administration, systemic disease, or infection. It was previously known as idiopathic thrombocytopenic purpura but is now known as immune thrombocytopenic purpura (ITP) for both idiopathic and secondary causes [7, 8].

33.3 Association Between Immune Thrombocytopenic Purpura and *H. pylori*

In 1998, Gasbarrini et al. [9] reported that *H. pylori* eradication resulted in an improved number of thrombocytes in 8 of 11 patients with autoimmune thrombocytopenia, and 6 of these 8 patients showed decreased autoantibodies, indicating a potential association between ITP and *H. pylori*. Following epidemiologic studies supported this idea. A nationwide population-based study of an Asian country showed that *H. pylori* infection was associated with increased risks of ITP in adults (odds ratio [OR] 5.93; 95% confidence interval [CI], 3.16–11.10) [10]. *H. pylori*-infected children had a lower average platelet count and mean platelet volume than uninfected children in a cross-sectional study of an African country (adjusted mean difference, -20.80×10^9/L; 95% CI, -33.51 to -8.09×10^9; $p = 0.001$, and adjusted mean difference, -0.236 fL; 95% CI, -0.408 to -0.065; $p = 0.007$, respectively) [11]. Increased thrombocyte levels after *H. pylori* eradication were reported by numerous studies, including a Korean study in which 17 (68%) of 25 patients with ITP showed complete or partial response [12] and a multicenter, open-label, prospective Korean study with *H. pylori*-positive patients ($n = 20$) with moderate thrombocytopenia ($30 \times 10^9/\text{L} \leq$ platelet count $\leq 70 \times 10^9/\text{L}$) also showed a complete response rate of 50% 3 months after the eradication treatment [13]; collectively, these findings support a causal relationship between ITP and *H. pylori* infection. A description of the studies reports increased levels of thrombocytes after *H. pylori* eradication on a regional basis. In 32 studies conducted with adults in Asia (23 in Japan, 2 in China, 2 in Iran, 3 in Korea, 1 in Pakistan, and 1 in Thailand), 1201 (71.1%) of 1688 patients with ITP were infected with *H. pylori*. Of the successfully eradicated 922 patients, 564 (61.2%) showed increased thrombocyte levels [13–19]. In Europe, in 10 studies conducted with adults (8 in Italy, 1 in Serbia, and 1 in Turkey), 288 (58.2%) of 495 patients with ITP were infected with *H. pylori*. Eradication treatment administered to 242 patients was successful in 222 patients (91.7%); of these patients, 108 (48.6%) showed increased levels of thrombocytes [14] (Table 33.1).

There have been few studies conducted with children. In three studies conducted in Asia (Japan, Iran, and Taiwan), 23.8% of the 63 patients were infected with *H. pylori*. The eradication rate in the 15 patients who received eradication treatment was 93.3% (14 patients), with seven (50%) patients showing a response in thrombocyte levels. In Europe, 21.1% of the 332 patients from four studies (two in Italy, one in the Netherlands, and one in Finland) were infected with *H. pylori*. Of these infected patients, 57 were administered eradication treatment, and the eradication rate was 93.0% (53 patients), with 29 patients (54.7%) showing a response in thrombocyte levels [14] (Table 33.1).

Because spontaneous resolution frequently occurs due to the nature of ITP and *H. pylori* is a common pathogen in the stomach, it is hard to determine the causal relationship between ITP and *H. pylori* infection based solely on the improvement in thrombocyte levels after *H. pylori* eradication. Macrolide-type antibiotics used for *H. pylori* eradication, such as clarithromycin, have an immune-regulatory effect and inhibit the production of pro-inflammatory cytokines, thereby improving the autoactivity of thrombocytes in ITP [20]. Alternatively, this could be the result of the elimination of other thrombocyte autoantibody-inducing bacteria in the body following the administration of antibiotics used to eradicate *H. pylori*. There are also reports claiming that the infection rate of *H. pylori* in patients with ITP is not different from that in the general public [21–25].

However, a systematic review of studies in which *Helicobacter* was eradicated not only in

Table 33.1 Association between *H. pylori* and immune thrombocytopenia [13–19]

	Continent	Country (no. of studies)	Patients with ITP (*n*)	*H. pylori*-infected ITP patients (*n*, %)	*H. pylori*-eradicated patients (*n*)	Patients with platelet response (*n*, %)
Adults	Total		2322	1544 (66.5%)	1184	703 (59.4%)
	Asia	Japan (23)	1688	1201 (71.1%)	922	564 (61.2%)
		China (2)				
		Korea (3)				
		Iran (2)				
		Pakistan (1)				
		Thailand (1)				
	Europe	Italy (8)	495	288 (58.2%)	222	108 (48.6%)
		Serbia (1)				
		Turkey (1)				
	America	Colombia (1)	139	55 (39.6%)	40	31 (77.5%)
		Brazil (1)				
		Canada (1)				
Children	Total		395	85 (21.5%)	67	36 (53.7%)
	Asia	Japan (1)	63	15 (23.8%)	14	7 (50.0%)
		Taiwan (1)				
		Iran (1)				
	Europe	Italy (2)	332	70 (21.1%)	53	29 (54.7%)
		Finland (1)				
		Netherlands (1)				

H. pylori Helicobacter pylori, *ITP* immune thrombocytopenic purpura patients with ITP infected with *H. pylori* but also in patients with ITP without *H. pylori* infection reported a significantly higher number of *H. pylori*-positive patients with increased thrombocyte levels than *H. pylori*-negative patients [26]. Only 8.8% of the patients with ITP who were *H. pylori*-negative had increased thrombocyte levels, compared with 51.2% of the patients with ITP who were *H. pylori*-positive (OR 14.5; 95% CI, 4.2–83.2) [26]. Furthermore, in a meta-analysis of only prospective studies, a higher number of thrombocytes were present with *H. pylori* eradication than with failed eradication or without eradication [27]. The weighted mean difference (WMD) of the thrombocyte levels for the group with *H. pylori* eradication was 52.2 (95% CI, 34.3–70.1; $p < 0.0001$) compared with the group with failed eradication and 40.8 (95% CI, 20.9–60.3; $p < 0.0001$) compared with the group without eradication, resulting in significant differences. Recently, two meta-analyses were reported. One report included the data of randomized trials from Japan, China, Thailand, and Brazil [28]. A total of 241 patients (125 in the eradication group and 116 in the control group) were included in the meta-analysis. Patients in the eradication group showed significantly higher overall platelet response rates than those in the control group (OR 1.93; 95% CI, 1.01–3.71; $p = 0\ 05$). Another report included only the data from the Middle East (Iran, Pakistan, and Turkey) [29]. A total of 228 *H. pylori*-infected patients (193 with successful eradication) were included in this study. If the OR was higher than 1, it was considered a better response for patients in the eradication group. The patients who received eradication treatment for *H. pylori* infection had a significantly higher OR (OR 8.83; 95% CI, 2.03–38.35; $p = 0.004$) than those in the non-eradicated group [29].

Owing to the strong evidence presented by the previously mentioned epidemiologic study of *H. pylori* infection with patients with ITP, the randomized controlled trials, systematic literature review, and meta-analysis of the changes in thrombocyte levels after *H. pylori* eradication treatment, many countries of Europe, North America (USA), South America (Brazil), and Asia (Korea and Japan) guidelines for *H. pylori* are recommending eradication of *H. pylori* for patients with ITP who have *H. pylori* infection [1–5].

33.4 Mechanisms

Because the underlying mechanism has not been determined, there are many hypotheses to explain the causal relationship between ITP and *H. pylori* infection. One hypothesis states that *H. pylori* cause nonspecific phagocytosis of monocytes and develops autoreactivity in B and T lymphocytes by reducing the inhibitory receptor FcγRII B of monocytes and inducing monocyte overexpression. It has been confirmed that this phenomenon is restored to the normal state once *H. pylori* is eradicated [30].

In addition, the production of autoantibodies that opsonize thrombocytes for destruction in the spleen might be associated with *H. pylori* infection. This hypothesis states that the antibody against *H. pylori*, especially against the cytotoxin-associated gene A (CagA) protein, causes cross-reaction with glycoproteins on the surfaces of thrombocytes such as glycoprotein IIIa. The evidence for this hypothesis is provided by a study reporting that these antibodies were decreased in some patients who showed cross-reaction between antibodies and CagA and complete recovery after *H. pylori* eradication. Another evidence is that there were many patients with CagA antibodies in a group that responded to eradication treatment than the nonresponded group (83% vs. 12.5%; $p = 0.026$) [31, 32]. There are differences in the improvement of thrombocyte levels with *H. pylori* eradication among regional reports, which could explain why *H. pylori* eradication was more effective in Japan, where there is a higher CagA-positive rate than in Western countries [33] (Table 33.1).

33.5 Conclusions

Although the exact mechanism has not been determined, epidemiologic studies, randomized controlled studies, and meta-analyses have reported an association between *H. pylori* and ITP and improved disease after *H. pylori* eradication. *H. pylori* eradication is highly recommended for patients with ITP who are infected with *H. pylori*.

References

1. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
2. Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG clinical guideline: treatment of *Helicobacter pylori* infection. Am J Gastroenterol. 2017;112:212–39.
3. Coelho LGV, Marinho JR, Genta R, Ribeiro LT, Passos MDCF, Zaterka S, et al. IVTH Brazilian consensus conference *Helicobacter pylori* infection. Arq Gastroenterol. 2018;55:97–121.
4. Jung HK, Kang SJ, Lee YC, Yang HJ, Park SY, Shin CM, et al. Evidence-based guidelines for the treatment of *Helicobacter pylori* infection in Korea 2020. Gut Liver. 2021;15:168–95.
5. Kato M, Ota H, Okuda M, Kikuchi S, Satoh K, Shimoyama T, et al. Guidelines for the management of *Helicobacter pylori* infection in Japan: 2016 revised edition. Helicobacter. 2019;24:e12597.
6. Neunert C, Terrell DR, Arnold DM, Buchanan G, Cines DB, Cooper N, et al. American Society of Hematology 2019 guidelines for immune thrombocytopenia. Blood Adv. 2019;3:3829–66.
7. Imbach P, Zimmerman S. Local and cultural aspects of childhood idiopathic thrombocytopenic purpura: a summary of statements from the 12 countries worldwide. J Pediatr Hematol Oncol. 2003;25 (Suppl 1):S68–73.
8. Rodeghiero F, Stasi R, Gernsheimer T, Michel M, Provan D, Arnold DM, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood. 2009;113:2386–93.
9. Gasbarrini A, Franceschi F, Tartaglione R, Landolfi R, Pola P, Gasbarrini G. Regression of autoimmune thrombocytopenia after eradication of *Helicobacter pylori*. Lancet. 1998;352:878.
10. Wu SR, Kuo HC, Huang WC, Huang YF, Chiou YH, Chang YH, et al. Incidence, clinical characteristics, and associated diseases in patients with immune thrombocytopenia: a nationwide population-based study in Taiwan. Thromb Res. 2018;164:90–5.
11. Baxendell K, Walelign S, Tesfaye M, Wordofa M, Abera D, Mesfin A, et al. Association between infection with *Helicobacter pylori* and platelet indices among school-aged children in Central Ethiopia: a cross-sectional study. BMJ Open. 2019;9:e027748.
12. Tag HS, Lee HS, Jung SH, Kim BK, Kim SB, Lee A, et al. Effects of *Helicobacter pylori* eradication in patients with immune thrombocytopenic purpura. Kor J Hematol. 2010;45:127–32.
13. Kim H, Lee WS, Lee KH, Bae SH, Kim MK, Joo YD, et al. Efficacy of *Helicobacter pylori* eradication for the 1st line treatment of immune thrombocytopenia patients with moderate thrombocytopenia. Ann Hematol. 2015;94:739–46.
14. Campuzano-Maya G. Hematologic manifestations of *Helicobacter pylori* infection. World J Gastroenterol. 2014;20:12818–38.
15. Sultan S, Irfan SM, Kaker J, Hasan M. Efficacy of *Helicobacter pylori* eradication as an upfront treatment of secondary immune thrombocytopenia: an experience from Pakistan. Med J Malaysia. 2016;71:53–6.
16. Sheema K, Ikramdin U, Arshi N, Farah N, Imran S. Role of *Helicobacter pylori* eradication therapy on platelet recovery in chronic immune thrombocytopenic purpura. Gastroenterol Res Pract. 2017;2017:9529752.
17. Treepongkaruna S, Sirachainan N, Kanjanapongkul S, Winaichatsak A, Sirithorn S, Sumritsopak R, et al. Absence of platelet recovery following *Helicobacter pylori* eradication in childhood chronic idiopathic thrombocytopenic purpura: a multi-center randomized controlled trial. Pediatr Blood Cancer. 2009;53:72–7.
18. Han B, Kim HJ, Yhim HY, Oh D, Bae SH, Shin HJ, et al. Sequential eradication of *Helicobacter pylori* as a treatment for immune thrombocytopenia in patients with moderate thrombocytopenia: a multicenter prospective randomized phase 3 study. Ann Hematol. 2022;101:1435–45.
19. Brito HS, Braga JA, Loggetto SR, Machado RS, Granato CF, Kawakami E. *Helicobacter pylori* infection & immune thrombocytopenic purpura in children and adolescents: a randomized controlled trial. Platelets. 2015;26:336–41.
20. Ianaro A, Ialenti A, Maffia P, Sautebin L, Rombola L, Carnuccio R, et al. Anti-inflammatory activity of macrolide antibiotics. J Pharmacol Exp Ther. 2000;292:156–63.
21. Liebman HA, Stasi R. Secondary immune thrombocytopenic purpura. Curr Opin Hematol. 2007;14:557–73.

22. Jarque I, Andreu R, Llopis I, De la Rubia J, Gomis F, Senent L, et al. Absence of platelet response after eradication of *Helicobacter pylori* infection in patients with chronic idiopathic thrombocytopenic purpura. Br J Haematol. 2001;115:1002–3.
23. Michel M, Khellaf M, Desforges L, Lee K, Schaeffer A, Godeau B, et al. Autoimmune thrombocytopenic purpura and *Helicobacter pylori* infection. Arch Intern Med. 2002;162:1033–6.
24. Michel M, Cooper N, Jean C, Frissora C, Bussel JB. Does *Helicobacter pylori* initiate or perpetuate immune thrombocytopenic purpura? Blood. 2004;103:890–6.
25. Estrada-Gómez RA, Parra-Ortega I, Martínez-Barreda C, Ruiz-Argüelles GJ. *Helicobacter pylori* infection and thrombocytopenia: a single-institution experience in Mexico. Rev Investig Clin. 2007;59:112–5.
26. Arnold DM, Bernotas A, Nazi I, Stasi R, Kuwana M, Liu Y, et al. Platelet count response to *H. pylori* treatment in patients with immune thrombocytopenic purpura with and without *H. pylori* infection: a systematic review. Haematologica. 2009;94:850–6.
27. Franchini M, Cruciani M, Mengoli C, Pizzolo G, Veneri D. Effect of *Helicobacter pylori* eradication on platelet count in idiopathic thrombocytopenic purpura: a systematic review and meta-analysis. J Antimicrob Chemother. 2007;60:237–46.
28. Kim BJ, Kim HS, Jang HJ, Kim JH. *Helicobacter pylori* eradication in idiopathic thrombocytopenic purpura: a meta-analysis of randomized trials. Gastroenterol Res Pract. 2018;2018:6090878.
29. Pezeshki SMS, Saki N, Ghandali MV, Ekrami A, Avarvand AY. Effect of *Helicobacter pylori* eradication on patients with ITP: a meta-analysis of studies conducted in the Middle East. Blood Res. 2021;56:38–43.
30. Asahi A, Nishimoto T, Okazaki Y, Suzuki H, Masaoka T, Kawakami Y, et al. *Helicobacter pylori* eradication shifts monocyte Fcgamma receptor balance toward inhibitory FcgammaRIIB in immune thrombocytopenic purpura patients. J Clin Invest. 2008;118:2939–49.
31. Scandellari R, Allemand E, Vettore S, Plebani M, Randi ML, Fabris F. Platelet response to *Helicobacter pylori* eradication therapy in adult chronic idiopathic thrombocytopenic purpura seems to be related to the presence of anticytotoxin-associated gene a antibodies. Blood Coagul Fibrinolysis. 2009;20:108–13.
32. Takahashi T, Yujiri T, Shinohara K, Inoue Y, Sato Y, Fujii Y, et al. Molecular mimicry by *Helicobacter pylori* CagA protein may be involved in the pathogenesis of *H. pylori*-associated chronic idiopathic thrombocytopenic purpura. Br J Haematol. 2004;124:91–6.
33. Sato R, Murakami K, Watanabe K, Okimoto T, Miyajima H, Ogata M, et al. Effect of *Helicobacter pylori* eradication on platelet recovery in patients with chronic idiopathic thrombocytopenic purpura. Arch Intern Med. 2004;164:1904–7.

34

Extraintestinal Manifestations of *H. pylori* Infection: Heart Disease

Seon Hee Lim

Abstract

Since the discovery that *Helicobacter pylori* (*H. pylori*) is a central player in the pathogenesis of gastric and duodenal ulcers, there have been many studies that demonstrate the role of *H. pylori* infection in extragastric diseases. New research is showing that it may also be linked to hyperlipidemia and heart diseases. However, there is still plenty of skepticism concerning cardiovascular diseases in which this widespread microbe may be implicated. Therefore, more studies, especially prospective randomized multicenter studies, are needed to illuminate the role of *H. pylori* infection in heart diseases.

Keywords

Coronary artery disease · Heart disease · *Helicobacter pylori* · Lipid profile

34.1 Introduction

Epidemiological and clinical studies have shown a possible link between *Helicobacter pylori* (*H. pylori*) infection and the risk of developing heart disease since the mid-1990s, but inhomogeneity in the study population and study methods along with potential confounders have yielded conflicting results.

The hypotheses of possible association between *H. pylori* infection and coronary heart diseases (CHD) are based on (1) the infection rate of *H. pylori* being higher in the group of CHD patients; (2) the association between the risk factors, such as blood lipid profile or fibrinogen, and *H. pylori* infection; (3) the positive correlation between C-reactive protein (CRP), which can be associated with CHD and the antibody of *H. pylori*; and (4) the presence of *H. pylori* in the atheroma [1].

Chronic infection of microorganisms accompanying inflammation was considered one of the major determinants in the pathogenesis of atherosclerosis [2–6]. The infection triggers a chronic inflammatory state along with other risk factors, such as dyslipidemia, hypercoagulability, and endothelial dysfunction, and contributes to the pathogenesis of atherosclerosis. Since Fabricant et al. [5] mentioned that infection may be related to atherosclerosis, several microbes have been reported as a causative microbe of atherosclerosis, and *H. pylori* is one of them. Mendall et al.

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[6] suggested the association of adult CHD with *H. pylori,* which was infected at the period of early childhood and kept lifelong seropositivity without eradication. Therefore, many scholars tried to reveal the role of *H. pylori* infection in the causation of myocardial infarction (MI) and obtained conflicting results.

The following issues will be discussed: (1) the relationship between *H. pylori* infection and its eradication and lipid profile; (2) the relationship between *H. pylori* infection and its eradication and atherosclerotic disease in the heart, i.e., CHD; and (3) the relationship between *H. pylori* infection and arrhythmia, i.e., atrial fibrillation (AF).

34.2 *H. pylori* and Lipid Profile

Since Gallin et al. [7] reported that systemic inflammatory response by infection of microbes caused lipid profile change, there have been many studies about the relation of *H. pylori* infection to lipid metabolism based on epidemiologic studies (Table 34.1) [8–23]. Among patients of CHD who were diagnosed by coronary angiography, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were not changed, but the high-density lipoprotein cholesterol (HDL-C) level was lower in the *H. pylori* seropositive group compared to the *H. pylori* seronegative group [15]. In another study, *H. pylori* infection was associated with higher triglycerides (TG) and lower HDL-C [24]. However, there were several studies that could not find the association between *H. pylori* infection and lipid profile [9, 11]. Among healthy persons, not among patients, LDL-C level was higher and HDL-C was lower in the *H. pylori* seropositive group compared with the seronegative group [16]. *H. pylori* infection is associated with lower HDL-C and higher TC, LDL-C, and TG levels. Higher apolipoprotein (apo)-B and lower apo-A levels were also reported [25].

The study results achieved so far about the association between lipid profile and *H. pylori* showed variable study designs, variable study objects, variable analytic methods, and then variable study results. The approximate assumption according to studies about the positive association between *H. pylori* and lipid profile is that *H. pylori* infection may cause elevation of TC, TG, LDL-C, and/or apo-B and decrease in HDL-C and/or apo-AI [8, 10, 12–18, 24]. These positive relations may be due to chronic infection of *H. pylori* that induces the secretion of cytokines related to chronic inflammation, such as interleukin (IL)-1, IL-6, interferon (IFN)-α, or tumor necrosis factor (TNF)-α, and then, these cytokines activate lipoproteinases in the fat tissue and synthesis of fatty acid and degradation of lipid in the liver [13, 26]. Otherwise, among studies so far there were also studies showing no relation between *H. pylori* infection and lipid profile [9, 11, 27].

The change in lipid profile after eradication also can tell the influence of *H. pylori* infection on lipid profile, but there were no opinions about the change after eradication. There have been many studies about the changes in lipid profile after eradication therapy of *H. pylori* infection (Table 34.2) [10, 11, 19, 21, 28–34].

Majka et al. [10] reported TC and LDL-C decreased in 6 months after *H. pylori* eradication, and Scharnagl et al. [28] also reported that patients who had duodenal ulcer showed that HDL-C, apo-AI, and apo-AII increase in 1 year after *H. pylori* eradication. Nam et al. [19] reported that successful eradication decreased the risk of high LDL-C and low HDL-C compared to that of persistent infection, whereas there were several studies that reported that TC and TG rather increased after *H. pylori* eradication [35–37]. Two meta-analysis studies [29, 31] on the changes in lipid profiles after *H. pylori* eradication showed different results; Upala et al. [29] reported that *H. pylori* eradication did not improve lipid profile, and on the other hand, Watanabe et al. [31] presented that *H. pylori* eradication increased HDL-C. Liou et al. [30] observed a significant reduction in TG and an increase in HDL-C at week 8 and 1 year after eradication therapies in multicenter, open-label, randomized trial. Park et al. [34] accounted that *H. pylori* eradication affected lipid profile differently depending on sex; HDL-C significantly

Table 34.1 Overview of investigations concerning the effects of HP infection on lipid profile

References	Study design	Objects	Test for HP infection	Results					Other remarks
				TC	LDL-C	HDL-C	TG	Apolipoproteins	
Lauria et al. (Finland, 1999) [8]	Single-center, cross-sectional	880 males (20–85y; mean 47y)	HP IgG, IgA	↑	NA	→	↑	NA	
Danesh et al. (UK, 1999) [9]	Case-control	1122 AMI pts (30–49y; mean 44y), 1122 age-sex-matched controls	HP IgG	→	NA	NA	NA	→	
Majka et al. (Germany, 2002) [10]	Case-control	80 stroke pts (60–75y), 80 age-sex-SES-matched controls	UBT	↑	↑	NA	NA	NA	
Elizalde et al. (Spain, 2002) [11]	Prospective multicenter cross-sectional	830 pts (mean 46.8y) with GI sx (+) and with EGD	CLO and/or UBT and/or histology	→	→	→	→	NA	
Rhee et al. (Korea, 2002) [12]	Single-center, cross-sectional	32,998 healthy persons (mean 40.6y)	HP IgG	↑	→	↓	→	→	In multiple logistic regression models, adjusted by age, sex, and other variables
Chimienti et al. (Italy, 2003) [13]	Single-center, cross-sectional	211 healthy males (27–70y; median 48y)	UBT, CagA IgG	↑	↑	→	→	NA	CagA(−) strains seem to have a major impact in modifying lipid levels than the CagA(+) ones
Sung et al. (Korea, 2005) [14]	Single-center, cross-sectional	58,981 healthy persons (mean 41.8y)	HP IgG	→	→	↓	↑	apo-AI (↓), apo-B (↑)	By multiple analysis of covariance analysis for HP infection
Jia et al. (China, 2009) [15]	Single-center, cross-sectional	961 CHD pts (54–70y; mean 63y) on coronary angiography	HP IgG	→	→	↓	→	NA	
Satoh et al. (Japan, 2010) [16]	Single-center, cross-sectional	6289 healthy persons (21–64y; mean 48y)	HP IgG	NA	↑	↓	→	NA	In men cf. no association with women

(continued)

Table 34.1 (continued)

References	Study design	Objects	Test for HP infection	Results					Other remarks
				TC	LDL-C	HDL-C	TG	Apolipoproteins	
Kim et al. (Korea, 2011) [17]	Single-center, cross-sectional	462 healthy persons (mean 66.2y) underwent EGD with gastric mucosal biopsy	Histology on gastric biopsy	→	↑	→	→	NA	Adjusted for age, sex, BMI, smoking, drinking, regular exercise, hypertension, diabetes, BP, fasting blood sugar, and creatinine
Lim et al. (Korea, 2013) [18]	Multicenter, cross-sectional	19,272 healthy persons (mean 50.3y)	HP IgG	↑	NA	NA	→	NA	
Nam et al. (Korea, 2015) [19]	Single-center, cross-sectional and longitudinal (prospective cohort study)	4269 health check-up persons	UBT	NA	↑	↓	→	NA	Adjusted for age, sex, BMI, smoking, drinking, and education duration
Kim et al. (Korea, 2016) [20]	Single-center, cross-sectional	37,263 healthy persons (mean 49.6y)	HP IgG	↑	↑	↓	↑	NA	
Adachi et al. (Japan, 2018) [21]	Single-center, cross-sectional	774 subjects (mean 52.6y)	HP IgG and EGD, with/without UBT or SAT	→	→	↓	→	NA	Adjusted for gender, age, BMI, smoking, and drinking
Shimamoto et al. (Japan, 2020) [22]	A combination of single-center, cross-sectional study	15,679 healthy persons (19–87y; mean 49y)	HP IgG	↑	↑	↓	↓	NA	Propensity score-based
	A meta-analysis	121,149 participants from 28 studies		↑	↑	↓	↑		
Haj et al. (Israel, 2021) [23]	A cross-sectional study using de-identified data from the computerized database of health maintenance organization	12,207 with diabetes (25–95y; mean 42.8y)	UBT	↑	↑	→	NA	NA	In unweighted multivariable analyses, adjusted for age, BMI, sex, SES, dyslipidemia, and use of statins and diabetes medications

→: no significant difference between the HP-positive and HP-negative groups; ↑: significantly higher in the HP-positive group than in the HP-negative group; ↓: significantly lower in the HP-positive group than in the HP-negative group

HP Helicobacter pylori; *TC* total cholesterol; *LDL-C* low-density lipoprotein cholesterol; *HDL-C* high-density lipoprotein cholesterol; *TG* triglyceride; *y* year(s); *NA* not applied; *AMI* acute myocardial infarction; *pts* patients; *SES* socioeconomic status; *UBT* urea breath test; *GI sx* gastrointestinal symptoms; *EGD* esophagogastroduodenoscopy; *CLO* Campylobacter-like organism; *apo* apoprotein; *CagA* cytotoxin-associated gene A; *CHD* coronary heart disease; *BMI* body mass index; *BP* blood pressure; *SAT* stool antigen test

Table 34.2 Overview of investigations concerning the effects of HP eradication on lipid profile

References	Study design	Objects	Test for HP eradication	Lipid profile FU time after HPE Tx	Results in the eradicated group					Other remarks
					TC	LDL-C	HDL-C	TG	Apolipoproteins	
Majka et al. (Germany, 2002) [10]	Case-control	80 stroke pts (60–75y), 80 age-sex-SES-matched controls	UBT	6m	↓	↓	NA	NA	NA	
Elizalde et al. (Spain, 2002) [11]	Prospective multicenter cross-sectional	830 Pts (mean 46.8y) with GI sx (+) and with EGD, and 466 pts with HPE Tx	CLO and/or UBT and/or histology	3m	↑	→	↑	↑	NA	After HPE Tx, minor increase in HDL-C, TC, and TG than the untreated group, but **this change is related to GI sx relief and lifestyle modification**, not to the outcome of HPE Tx
Scharnagl et al. (Austria, 2004) [28]	Observational, longitudinal study	87 duodenal ulcer pts (mean 52y)	CLO, histology, UBT	1y	↑	→	↑	↑	↑ in apo-AI, ↑ in apo-AII, ↑ in apo-B	
Nam et al. (Korea, 2015) [19]	Single-center, cross-sectional, and longitudinal (prospective cohort study)	4269 health check-up persons 529 HPE Tx with success	UBT, CLO, or histology	1–3y	NA	↓	↑	→	NA	By HPE Tx Adjusted OR for ↓ risk of high LDL-C: 0.76 (0.59–0.96) ↓ risk of low HDL-C: 0.68 (0.49–0.96)
Upala et al. (USA & Thailand, 2017) [29]	Systemic review and meta-analysis	934 participants (32.5–60.4y; mean 50.5y) from five studies	UBT, CLO, and/or histology	6–12w	NA	→	→	→	NA	
Adachi et al. (Japan, 2018) [21]	Single-center, cross-sectional, and longitudinal	774 subjects (mean 52.6y)	UBT or SAT	2y	↓	↓	↑	↓	NA	But no significant change between HP-eradicated and HP-persistent groups after adjusting for gender, age, BMI, smoking, and drinking

(continued)

Table 34.2 (continued)

References	Study design	Objects	Test for HP eradication	Lipid profile FU time after HPE Tx	Results in the eradicated group					Other remarks
					TC	LDL-C	HDL-C	TG	Apolipoproteins	
Liou et al. (Taiwan, 2019) [30]	Multicenter, open-label, randomized trial	1620 participants (mean 53y), randomly assigned to three treatment groups	*Detection* by histology, culture, CLO, and serology (two positive tests among them) *Eradication* by UBT	8w	↑$^{\#}$	↑$^{\#}$	↑	↓$^{\#}$	NA	$^{\#}$In concomitant therapy and quadruple therapy groups *In concomitant therapy group
				1y	↑*	↑*	↑	↓*		
Watanabe et al. (Japan, 2021) [31]	A meta-analysis	5270 participants (median 48.4y) from 24 studies including 4 RCTs and 20 non-RCTs	HP IgG, UBT, CLO, histology, culture, and/or SAT	0.08 to <60 m	→	→	↑	↑	NA	In the analyses of RCTs only, elevated HDL-C, but not TG
Park et al. (Korea, 2021) [32]	Single-center, cohort study	2626 healthy participants without dyslipidemia	*Detection* by histology *Eradication* by UBT	Median 3.5y (2–5.9y)	↓	↓	↑	↓	NA	HR for high TC: 0.92 (0.84–0.99), for low HDL-C: 0.63 (0.47–0.85) in women, cf. 0.91 (0.79–1.05) in men
Wang et al. (China, 2022) [33]	Single-center, cohort	3412 matched cases (mean 45.3y)	UBT	1.5y	→	↑	↓	↑	NA	Propensity score-matched study
Park et al. (Korea, 2022) [34]	Single-center, prospective observational cohort study	2267 pts with GI sx (+) underwent EGD	Histology, culture, or CLO	1,3,5 y	→	→	↑	→	NA	In women cf. no association in men

→: no difference between the HP-eradicated and non-HP-eradicated groups; ↑: higher in the HP-eradicated group than in the non-HP--eradicated group; ↓: lower in the HP-eradicated group than in the non-HP-eradicated group

HP Helicobacter pylori; *FU* follow-up; *HPE Tx* HP eradication therapy; *TC* total cholesterol; *LDL-C* low-density lipoprotein cholesterol; *HDL-C* high-density lipoprotein cholesterol; *TG* triglyceride; *pts* patients; *y* year(s); *SES* socioeconomic status; *UBT* urea breath test; *m* month(s); *NA* not applied; *GI sx* gastrointestinal symptoms; *EGD* esophagogastroduodenoscopy; *CLO* Campylobacter-like organism; *apo* apolipoprotein; *OR* odds ratio; *CI* confidence interval; *w* week(s); *SAT* stool antigen test; *BMI* body mass index; *RCT* randomized controlled trial; *HR* hazard ratio increased only in females over time up to 5 years of follow-up.

In this way, the change in lipid profile by *H. pylori* infection or *H. pylori* eradication could not show a constant trend. The association between lipid profile and *H. pylori* has been increasingly discussed but still remains unclear. Therefore, the study will need to show concrete evidence that *H. pylori* infection influences lipid profile.

34.3 *H. pylori* and Coronary Artery Disease

Recent studies suggested a possible link between *H. pylori* infection and CHD, but overall the association between *H. pylori* and coronary artery disease is not strong and is still debated, so a well-designed prospective randomized blind study is yet to be needed. Already-published studies seem to explore three aspects: (1) the relation between ischemic heart disease and *H. pylori* infection by serology or histology; (2) the relation between ischemic heart disease and cytotoxin-associated gene A (CagA) antibody; and (3) the relation between recurrence of ischemic heart disease and eradication of *H. pylori*. The results of studies about *H. pylori* and coronary artery disease are summarized in Table 34.3 [6, 9, 38–58].

Although some seroepidemiological or clinical studies demonstrated associations between CHD and antibody to *H. pylori* [6, 9, 19, 40, 41, 44, 45, 49, 50, 52–56], other studies did not [24, 38, 39, 42, 43, 46, 47, 51]. The results are still inconsistent. Since the mid-2010s, there have been several meta-analysis studies on the relationship between *H. pylori* infection and CHD [53–56], and they showed possible indication of the relationship between the two. Mach et al. [59] conducted an in vivo study about a mouse model of atherosclerosis, but there was no indication that the *H. pylori* infection might contribute to the development of atherosclerotic lesion formation. The change in incidence/prevalence of CHD or anatomical or functional aspect of CHD after *H. pylori* eradication also can show the effect of *H. pylori* infection on CHD. Wang et al. [57] carried out a cohort study using big data from the Taiwan National Health Insurance Research Database and reported time-dependent decreased association between *H. pylori* eradication and CHD. Kim et al. [58] manifested that *H. pylori* eradication prevented CHD and this effect was different depending on age and sex; *H. pylori* eradication prevented CHD in males ≤65 years and females >65 years, but not in males aged >65 years and females ≤65 years. There was a study [60] that reported that the endothelial function of vessels was improved after *H. pylori* was eradicated or another study [61] that showed that the recurrence of CHD was decreased after eradication therapy of *H. pylori*, while there was a study that reported no difference in recurrence of CHD between with and without eradication of *H. pylori* [62].

There is no definite theory yet about the mechanisms that *H. pylori* infection causes atherosclerosis, but we can get the presumed pathogenic mechanisms based on the results of many studies; *H. pylori* may secrete cytotoxin and activate inflammatory cytokines, such as TNF-α, IL-6, or fibrinogen, and then induce chronic inflammatory cascades and change lipid metabolism, and these changes may contribute to the development of atherosclerosis [49, 63, 64]. Besides inflammatory reactions by *H. pylori*, circulating antibodies to the heat shock protein (HSP) 60 family in patients with *H. pylori* infection have been reported [65]. This *H. pylori* HSP may contribute to endothelial damage by inducing anti-*H. pylori* HSP antibodies that cross-react with host HSP because of the homology between *H. pylori* HSP with the human HSP and induce immunopathology by molecular mimicry [65–67]. Kountouras et al. [68] presumed that monocytes secrete tissue factors with procoagulant activity by *H. pylori* infection, and these factors activate coagulation cascades and then cause CHD. However, this hypothesis is also unclear because there is a contrary result [69].

Chronic infection with *H. pylori* might result in a low-grade acute-phase response and increased levels of acute-phase coagulation proteins; in particular, fibrinogen is a strong predictor of CHD [70, 71]. This may provide a link between infection and acute thrombotic events. Patel et al. [72] supported this suggestion, where

Table 34.3 Overview of investigations concerning the effects of HP infection/eradication on CHD prevalence

References	Study design	Objects	Test for HP infection	Results	Other remarks
Mendell et al. (UK, 1994) [6]	Single-center, case-control	111 CHD pts, 74 controls	HP IgG (ELISA)	*Associated* OR adjusted for confounding factors (2.15 (1.07–4.29))	OR adjusted for confounding factors and childhood environmental factors (1.90 (0.91–3.97))
Murray et al. (UK, 1995) [38]	Cross-sectional	1182 men, 1198 women	HP IgG (ELISA)	*Not associated* OR adjusted for confounding factors (1.51 (0.93–2.45))	
Folsom et al. (USA, 1998) [39]	Prospective, case-cohort (FU 3.3y)	498 cohort samples, 217 incident of CHD	HP IgG (ELISA)	*Not associated* Adjusted HR for confounding factors (1.03 (0.68–1.57))	
Pasceri et al. (Italy, 1998) [40]	Single-center, case-control	88 IHD pts on the angiography, 88 age-sex-matched controls	HP IgG (ELISA), CagA IgG (Western blot)	*Associated* OR adjusted for confounding factors (2.8 (1.3–7.4))	↑ CAD risk in CagA(+): OR adjusted for confounding factors (3.8 (1.6–9.1))
Danesh et al. (UK, 1999) [9]	Case-control	1122 AMI pts, 1122 age-sex-matched controls	HP IgG (ELISA)	*Associated* OR adjusted for confounding factors (1.75 (1.29–2.36))	
Pellicano et al. (France, 1999) [41]	Meta-analysis of 24 studies	6603 sample size	HP IgG (ELISA) or UBT	Possible weak association Pooled OR 1.55 (1.38–1.74)	
Whincup et al. (UK, 2000) [42]	Multicenter, prospective, nested case-control (FU 9.5y)	505 CHD pts, 1026 controls	HP IgG (ELISA) CagA IgG (ELISA)	*Not associated* OR adjusted for confounding factors (1.3 (0.88–1.90))	Not ↑ CAD risk in CagA(+): OR adjusted for confounding factors (1.1 (0.17–1.71))
Zhu et al. (USA, 2002) [43]	Study 1: cross-sectional Study 2: single-center, prospective longitudinal (FU 3y)	Study 1: 391 pts who angiography → 63%, CAD Study 2: 929 CAD on angiography	HP IgG (ELISA)	*Not associated* Study 1: OR adjusted for confounding factors (1.03 (0.60–1.77)) Study 2: HR adjusted for HP IgG(+) (1.12 (0.81–1.54))	
Kinjo et al. (Japan, 2002) [44]	Multicenter, prospective, case-control	618 AMI pts, 967 controls	HP IgG (ELISA)	*Not associated* Overall OR adjusted for clinical factors 0.97 (0.71–1.32)	*Associated* In subgroup below 55 y: OR adjusted for clinical factors (2.97 (1.37–6.41))
Fraser et al. (New Zealand, 2003) [45]	Case-control	341 AMI pts by the registry, 831 community controls	HP IgG (ELISA)	*Associated* OR adjusted for age and sex (1.34 (1.00–1.80)) ($p = 0.038$)	
Sheehan et al. (Ireland, 2005) [46]	Community-based case-control	227 ACS pts, 277 age-sex-matched controls	HP IgG (ELISA)	*Not associated* OR adjusted for confounding factors (0.9 (0.8–1.0))	

Table 34.3 (continued)

References	Study design	Objects	Test for HP infection	Results	Other remarks
Jin et al. (Korea, 2007) [47]	Single-center, prospective, case-control	175 CAD on angiography, 88 controls with normal angiography	Histology	*Not associated* No difference in HP infection rate between CAD pts and controls (40.6% vs. 30.7%, ns), post-PCI re-intervention rate, and formation of new coronary lesions, respectively	
Zhang et al. (China, 2008) [48]	Meta-analysis of 15 case-control studies	2157 CHD pts, 2383 controls	CagA IgG (ELISA or Western blot)	*CagA (+) infection is associated with susceptibility to CHD* OR (random) 2.11 (1.70–2.62)	
Tamer et al. (Turkey, 2009) [49]	Single-center, case-control	152 CAD pts (73 ACS, 79 SA), 22 controls	HP IgG (ELISA)	*Associated* ↑prevalence of HP IgG in CAD pts (80.2% vs. 54.4%, $p = 0.015$)	No difference in HP IgG (+) rate between ACS and SA
Khodaii et al. (Iran, 2011) [50]	Single-center, case-control	500 AMI pts on angiography, 500 controls	HP IgG (ELISA) CagA IgG (ELISA and Western blot)	*Associated* Crude OR 2.57 (1.89–3.49)	AMI risk in CagA(+): crude OR 1.67 (1.18–2.36)
Schöttker et al. (Germany, 2012) [51]	Population-based cohort (FU 5.1y)	9953 participants →170 AMI (+)	HP IgG (ELISA), CagA IgG (ELISA)	*Not associated* Adjusted HR for confounding factors (0.70 (0.46–1.08))	CagA(+): adjusted HR for confounding factors (1.09 (0.76–1.57))
Lai et al. (Taiwan, 2014) [52]	A nationwide retrospective cohort (using the Taiwan National Health Insurance Research database)	17,075 HP-infected cases, 68,300 age-sex-matched controls	ICD-9-CM 041.86 (HP infection), ICD-9-CM 410, 411.1, 411.8 (ACS)	*Associated* Adjusted HR for confounding factors (1.48 (1.30–1.69))	
Nam et al. (Korea, 2015) [19]	Single-center, cross-sectional, and longitudinal (prospective cohort study)	4269 health check-up persons	UBT, CLO, or histology	*Associated* OR 3.42 (1.3–8.61)	Adjusted for age, sex, BMI, smoking, drinking, diabetes, hypertension, LDL-C, HDL-C, TG, and education duration
		529 HPE Tx with success, 1806 persistent HP infection, 1934 persistent HP (−)		HPE Tx *did not decrease* the risk of CHD at FU	
Liu et al. (China, 2015) [53]	A meta-analysis	5829 MI pts and 16,131 controls from 26 studies	HP IgG	*Associated* OR 2.10 (1.75–2.53)	– No age and race differences – MI with CagA strain; lack of data

(continued)

Table 34.3 (continued)

References	Study design	Objects	Test for HP infection	Results	Other remarks
Sun et al. (USA, 2016) [54]	A meta-analysis	22,207 participants from 19 studies	HP IgG	*Associated* RR 1.11 (1.01–1.22) cf. ≥ 10y ($n = 5100$, RR 1.04 [0.87–1.24])	– This association was weaker in long-term FU – CagA strain: no association with MI
Wang et al. (China, 2021) [55]	A meta-analysis	19,691 participants from 40 studies		*Associated* OR 1.51 (1.34–2.70) – MI: OR 1.80 (1.42–2.26) – IHD: OR 1.34 (1.12–1.60)	HP infection with CagA(+) could increase CVDs: combined effect size was OR 1.73 (1.40–2.14) with heterogeneity between studies was moderate ($I^2 = 65.4\%$, $p = 0.001$) and slight publication bias
Tong et al. (China, 2022) [56]	A meta-analysis	18,046 CHD pts and 12,879 controls from 44 studies (16,236 participants from 13 prospective studies and 14,689 participants from 31 cross-sectional studies)	HP IgG, CagA IgG, SAT, histology	*Associated with CHD* – HP IgG: OR 1.58 (1.34–1.87) – Anti-CagA: OR 1.33 (1.16–1.53) – SAT: OR 3.50 (1.60–7.66) – Histology: OR 1.78 (1.12–2.83)	
Wang et al. (Taiwan, 2018) [57]	A cohort study using big data from the Taiwan National Health Insurance Research Database	Among 58,413 PUD pts, 3164 PUD pts with HPE Tx and 3164 age-sex-comorbidity-matched PUD without HPE Tx *Early HPE Tx: ≤90 days after index date		*Decreased association of CHD* in pts with early *HPE Tx* compared to those without HPE Tx (2.58% vs. 3.35%, $p = 0.09$)	– Propensity score-based – Time-dependent association. The mortality rate was lower in the early HPE Tx subgroup compared to those without HPE Tx (2.86% vs. 4.43%, $p = 0.0033$)
Kim et al. (Korea, 2022) [58]	Single-center, longitudinal study	4765 subjects – 3783 pts with HPE Tx and 982 pts without HPE Tx – FU 67.5 m	*Detection* by histology, CLO, or culture *Eradication* by UBT, histology, and/or CLO at FU (1 or 2y)	HPE Tx *prevented CHD* in males ≤65y: HR 0.133 (0.039–0.455, $p = 0.001$) and females aged >65y: HR 0.260 (0.110–0.615, $p = 0.002$)	– Propensity score-based – No association in male >65y and female ≤65y

HP Helicobacter pylori; *CHD* coronary heart disease; *pts* patients; *ELISA* enzyme-linked immunosorbent assay; *OR* odds ratio; *FU* follow-up; *y* year(s); *HR* hazard ratio; *IHD* ischemic heart disease; *CagA* cytotoxin-associated gene A; *CAD* coronary artery disease; *AMI* acute myocardial infarction; *PCI* percutaneous coronary intervention; *UBT* urea breath test; *ACS* acute coronary syndrome; *SA* stable angina; *ns* nonsignificant; *ICD-9-CM* International Classification of Diseases; Ninth Revision; Clinical Modification; *BMI* body mass index; *LDL-C* low-density lipoprotein cholesterol; *HDL-C* high-density lipoprotein cholesterol; *TG* triglycerides; *MI* myocardial infarction; *RR* risk ratio; *PUD* peptic ulcer disease; *HPE Tx H. pylori* eradication therapy; *m* month(s); *OR* odds ratio elevated fibrinogen levels were independently associated with *H. pylori* infection compared with those free from infection.

There is another presumption that *H. pylori* may induce platelet aggregation and then induce instability of atherosclerosis [73, 74]. Vijayvergiya and Vadivelu [75] summarized the theory of the atherogenic capacity of *H. pylori* infection for multiple pathways, including platelet aggregation. The possible mechanisms of *H. pylori* are related to atherosclerotic change are [75]:

1. Induction of inflammatory response secondary to chronic infectious state
2. Endothelial damage
3. Chronic low-grade activation of coagulation cascade
4. Dysregulation of lipid metabolism resulting in increased TC and TG levels and reduced HDL-C levels
5. Hyperhomocysteinemia

The cytotoxic strains of *H. pylori* bearing the CagA induce a stronger inflammatory and systemic immune response and are associated with increased inflammation in the development of atherothrombosis [76, 77]. The possible importance of CagA in vascular diseases is also supported by evidence of cross-reactivity of anti-CagA antibodies with arterial wall antigens, suggesting a possible mechanism enhancing vascular inflammation and atherosclerosis [78].

Several case-control and cohort studies assessed the association of CagA status with atherosclerotic diseases and reported that the patients with atherothrombotic disease seemed more likely to be infected by the CagA-seropositive strains of *H. pylori* than healthy subjects [9, 48, 50, 79, 80].

A recent meta-analysis showed that infection sustained by those strains may be considered a marker of vascular damage for both coronary and cerebral vessels [80]. The mechanisms underlying this pro-atherosclerotic effect are probably different. Molecular mimicry between CagA antigen and peptides localized in endothelial cells, smooth muscle cells, and macrophages of both normal and atherosclerotic arteries has already been shown [78].

CagA-positive strains of *H. pylori* have also been found to play a role in the destabilization of coronary plaques, and a meta-analysis has also shown a significant association between infection sustained *by CagA*-positive strains of *H. pylori* and the occurrence of acute coronary syndromes [81, 82]. However, there are also studies that could not elicit the association between CagA-positive *H. pylori* and CHD [42, 51].

34.4 *H. pylori* and Arrhythmia

In 1997, Frustaci et al. [83] reported their observation of inflammatory infiltrates, myocyte necrosis, and fibrosis in atrial biopsies of patients with lone AF refractory to antiarrhythmic drug therapy. More and more evidence has shown that inflammatory reaction has been closely related to AF since 1997. The concept that inflammation contributes to at least some types of AF came from studies that showed that the frequent occurrence (25–40%) of AF after cardiac surgery and the temporal course of AF occurring after cardiac surgery closely follow the activation of the complement system and release of pro-inflammatory cytokines [84–86].

The CRP levels were higher in patients with AF than in a control group of patients with normal sinus rhythm, and CRP also predicted patients at increased risk of developing future AF [86–88]. Montenero et al. [89] reported that some patients with *H. pylori* have autoantibodies to the Na^+/K^+-ATPase, the proton pump of gastric parietal cells, which is similar to Na^+/K^+-ATPase, the pump of cardiac cells. In fact, cardiac Na^+/K^+-ATPase and Na^+/K^+-ATPase have a similar 35 kDa glycoprotein necessary for their catalytic activity. They hypothesized that these autoantibodies to Na^+/K^+-ATPase may also bind to Na^+/K^+-ATPase, thus determining atrial damage, and the role of these pumps is to maintain ionic homeostasis by hydrolyzing ATP, and therefore, loss of this balance may trigger AF by determining abnormal automaticity or triggered activity that causes delay after depolarization inducing very rapid premature atrial contractions.

So far, there were some studies showing that chronic *H. pylori* infection was associated with AF and contributed to the occurrence of AF [89–93], but others reported that *H. pylori* infection was not related to AF [94–96]. The results of studies about the relation of *H. pylori* infection to arrhythmia are summarized in Table 34.4 [89, 93–99].

Table 34.4 Overview of investigations concerning the effects of HP infection on cardiac arrhythmia

References	Study design	Objects	Test for HP infection	Results	Other remarks
Montenero et al. (Italy, 2005) [89]	Single-center, case-control	59 pts with a paroxysmal or persistent AF, 45 healthy controls	HP IgG (ELISA)	HP seropositivity in AF is higher than that in controls (97.2 IU/mL vs. 5.3 IU/mL, $p < 0.001$)	HP seropositivity in persistent AF is higher than that in paroxysmal AF ($p = 0.027$) CRP in AF is higher than that in controls ($p < 0.001$)
Bunch et al. (USA, 2008) [93]	Single-center, case-control	83 AF pts, 660 controls	HP IgG (ELISA)	HP seropositivity in AF is higher than that in controls (65% vs. 55%, $p = 0.049$)	
Platonov et al. (Sweden, 2008) [94]	Single-center, case-control	72 permanent AF, 72 age- and sex-matched controls	HP IgG (EIA)	No difference in HP seropositivity between AF and controls (57% vs. 55%, ns)	CRP in AF is higher than that in controls ($p < 0.001$)
Lunetta et al. (Italy, 2009) [95]	Prospective FU 7y	120 HP(+), 60 HP(−)	HP IgG	No difference in development of AF between HP(+) vs. HP(−) (21% vs. 18%, ns)	
Franceschi et al. (Italy, 2013) [96]	Single-center case-control	54 idiopathic dysrhythmia, 50 controls	UBT, CagA IgG (Western blot)	No difference in HP IgG(+) between pts vs. controls (42% vs. 44%, ns)	CagA IgG(+) in pts is higher than that in controls (65% vs. 42%, $p < 0.01$)
Wang et al. (China, 2015) [97]	Single-center case-control	285 hospitalized pts with AF, 300 age-sex-matched controls	UBT	HP value in pts with long-standing AF were higher than those in short-standing AF and the groups ($p < 0.001$ for both, respectively).	hs-CRP level in pts with long-standing AF was higher than those in short-standing AF and control groups ($p = 0.003$, 0.002, respectively)
Yan et al. (China, 2016) [98]	A meta-analysis	675 arrhythmia pts and 1339 controls from 7 studies		HP positivity: 58.1% in arrhythmia group vs. 47.8% in controls Pooled OR 1.8 (1.08–2.99); test for overall effect $Z = 2.26$ ($p = 0.024$)	There may be a relationship between HP infection and risk of arrhythmia. In subgroup analysis, HP infection was a risk factor for AF in Asia and Africa

Table 34.4 (continued)

References	Study design	Objects	Test for HP infection	Results	Other remarks
Tetta et al. (The Netherlands, 2019) [99]	Systemic review and meta-analysis	956 AF pts and 1965 controls from 6 articles	Histology, culture, PCR, CLO, UBT, serology, or SAT	HP positivity: 35% in AF vs. 32.7% in controls ($p = 0.21$), cumulative risk ratio for AF for HP infection 1.19 (1.08–1.41)	A weak correlation between AF and HP (coefficient = 0.04 [0.01–0.08]) with significant heterogeneity in the selected studies ($I^2 = 48\%$, $p = 0.02$) neither RR nor risk difference was influenced by the geographic area

HP Helicobacter pylori; *pts* patients; *AF* atrial fibrillation; *CRP* C-reactive protein; *ELISA* enzyme-linked immunosorbent assay; *EIA* enzyme immunoassay; *ns* nonsignificant; *FU* follow-up; *y* year(s); *UBT* urea breath test; *CagA* cytotoxin-associated gene A; *SAT* stool antigen test; *OR* odds ratio

34.5 Conclusions

So far, published studies have used various methods for the diagnosis of *H. pylori*. The easiest and rapidest, so available for large-scale test, serologic test for *H. pylori* was used in most of studies, but there were few studies that used a diagnostic method, which can evaluate active infection. Moreover, there are a lot of confounding factors such as socioeconomic factors, smoking, and genetic backgrounds to consider. Therefore, the role of *H. pylori* in CHD is still conflicting.

Investigation about the causal relationship between *H. pylori* and CHD may be very important in the aspect of public health, in countries where *H. pylori* is more prevalent, and cardiovascular diseases are the leading cause of death, such as Korea, China, or India.

A prospective randomized multicenter study may be needed to establish the relationship between two diseases and, by extension, the causal-and-effect relationship. In particular, a prospective population-based randomized interventional study about the influence of *H. pylori* eradication on the prevention of primary or secondary occurrence of CHD may be solving clues.

References

1. Kowalski M, Rees W, Konturek PC, Grove R, Scheffold T, Meixner H, et al. Detection of *Helicobacter pylori* specific DNA in human atheromatous coronary arteries and its association to prior myocardial infarction and unstable angina. Dig Liver Dis. 2002;34:398–402.
2. Markle HV. Coronary artery disease associated with *Helicobacter pylori* infection is at least partially due to inadequate folate status. Med Hypotheses. 1997;49:289–92.
3. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med. 1999;340:115–26.
4. Gupta S. Chronic infection in the aetiology of atherosclerosis—focus on chlamydia pneumoniae. Atherosclerosis. 1999;143:1–6.
5. Fabricant CG, Fabricant J, Litrenta MM, Mink CR. Virus-induced atherosclerosis. J Exp Med. 1978;148:335–40.
6. Mendall MA, Goggin PM, Molineaux N, Levy J, Toosy T, Strachan D, et al. Relation of *Helicobacter pylori* infection and coronary heart disease. Br Heart J. 1994;71:437–9.
7. Gallin JI, Kaye D, O'Leary WM. Serum lipids in infection. N Engl J Med. 1969;281:1081–6.
8. Laurila A, Bloigu A, Näyhä S, Hassi J, Leinonen M, Saikku P. Association of *Helicobacter pylori* infection with elevated serum lipids. Atherosclerosis. 1999;142:207–10.
9. Danesh J, Youngman L, Clark S, Parish S, Peto R, Collins R. *Helicobacter pylori* infection and early

onset myocardial infarction: case-control and sibling pairs study. BMJ. 1999;319:1157–62.

10. Majka J, Róg T, Konturek PC, Konturek SJ, Bielański W, Kowalsky M, et al. Influence of chronic *Helicobacter pylori* infection on ischemic cerebral stroke risk factors. Med Sci Monit. 2002;8:CR675–84.
11. Elizalde JI, Piqué JM, Moreno V, Morillas JD, Elizalde I, Bujanda L, et al. Influence of *Helicobacter pylori* infection and eradication on blood lipids and fibrinogen. Aliment Pharmacol Ther. 2002;16:577–86.
12. Rhee EJ, Sung KC, Kim BS, Kang JH, Lee MH, Park JR, et al. Prevalence of *Helicobacter pylori* infection and its association with cardiovascular risk factors in Korean adults. Korean J Med. 2002;63:469–76.
13. Chimienti G, Russo F, Lamanuzzi BL, Nardulli M, Messa C, Di Leo A, et al. *Helicobacter pylori* is associated with modified lipid profile: impact on lipoprotein(a). Clin Biochem. 2003;36:359–65.
14. Sung KC, Rhee EJ, Ryu SH, Beck SH. Prevalence of *Helicobacter pylori* infection and its association with cardiovascular risk factors in Korean adults. Int J Cardiol. 2005;102:411–7.
15. Jia EZ, Zhao FJ, Hao B, Zhu TB, Wang LS, Chen B, et al. *Helicobacter pylori* infection is associated with decreased serum levels of high density lipoprotein, but not with the severity of coronary atherosclerosis. Lipids Health Dis. 2009;8:59–65.
16. Satoh H, Saijo Y, Yoshioka E, Tsutsui H. *Helicobacter pylori* infection is a significant risk for modified lipid profile in Japanese male subjects. J Atheroscler Thromb. 2010;17:1041–8.
17. Kim HL, Jeon HH, Park IY, Choi JM, Kang JS, Min KW. *Helicobacter pylori* infection is associated with elevated low density lipoprotein cholesterol levels in elderly Koreans. J Korean Med Sci. 2011;26:654–8.
18. Lim SH, Kwon JW, Kim N, Kim GH, Kang JM, Park MJ, et al. Prevalence and risk factors of *Helicobacter pylori* infection in Korea: nationwide multicenter study over 13 years. BMC Gastroenterol. 2013;13:104–13.
19. Nam SY, Ryu KH, Park BJ, Park S. Effects of *Helicobacter pylori* infection and its eradication on lipid profiles and cardiovascular diseases. Helicobacter. 2015;20:125–32.
20. Kim TJ, Lee H, Kang M, Kim JE, Choi Y-H, Min YW, et al. *Helicobacter pylori* is associated with dyslipidemia but not with other risk factors of cardiovascular disease. Sci Rep. 2016;6:1–8.
21. Adachi K, Mishiro T, Toda T, Kano N, Fujihara H, Mishima Y, et al. Effects of *Helicobacter pylori* eradication on serum lipid levels. J Clin Biochem Nutr. 2018;62:264–9.
22. Shimamoto T, Yamamichi N, Gondo K, Takahashi Y, Takeuchi C, Wada R, et al. The association of *Helicobacter pylori* infection with serum lipid profiles: an evaluation based on a combination of meta-analysis and a propensity score-based observational approach. PLoS One. 2020;15:e0234433.
23. Haj S, Chodick G, Goren S, Na'amnih W, Shalev V, Muhsen K. Differences in glycated hemoglobin levels and cholesterol levels in individuals with diabetes according to *Helicobacter pylori* infection. Sci Rep. 2021;11:8416.
24. Niemela S, Karttunen T, Korhonen T, Läärä E, Karttunen R, Ikäheimo M, et al. Could *Helicobacter pylori* infection increase the risk of coronary heart disease by modifying serum lipid concentrations? Heart. 1996;75:573–5.
25. Hoffmeister A, Rothenbacher D, Bode G, Persson K, März W, Nauck MA, et al. Current infection with *Helicobacter pylori*, but not seropositivity to *chlamydia pneumoniae* or cytomegalovirus, is associated with an atherogenic, modified lipid profile. Arterioscler Thromb Vasc Biol. 2001;21:427–32.
26. Feingold KR, Grunfeld C. Role of cytokines in inducing hyperlipidemia. Diabetes. 1992;41 (Suppl 2):97–101.
27. Regnstrom J, Jovinge S, Bavenholm P, Ericsson CG, De Faire U, Hamsten A, et al. *Helicobacter pylori* seropositivity is not associated with inflammatory parameters, lipid concentrations and degree of coronary artery disease. J Intern Med. 1998;243:109–13.
28. Scharnagl H, Kist M, Grawitz AB, Koenig W, Wieland H, März W. Effect of *Helicobacter pylori* eradication on high-density lipoprotein cholesterol. Am J Cardiol. 2004;93:219–20.
29. Upala S, Sanguankeo A, Saleem SA, Jaruvongvanich V. Effects of *Helicobacter pylori* eradication on insulin resistance and metabolic parameters: a systematic review and meta-analysis. Eur J Gastroenterol Hepatol. 2017;29:153–9.
30. Liou JM, Chen CC, Chang CM, Fang YJ, Bair MJ, Chen PY, et al. Long-term changes of gut microbiota, antibiotic resistance, and metabolic parameters after *Helicobacter pylori* eradication: a multicentre, open-label, randomised trial. Lancet Infect Dis. 2019;19:1109–20.
31. Watanabe J, Hamasaki M, Kotani K. The effect of *Helicobacter pylori* eradication on lipid levels: a meta-analysis. J Clin Med. 2021;10:904.
32. Park Y, Kim TJ, Lee H, Yoo H, Sohn I, Min YW, et al. Eradication of *Helicobacter pylori* infection decreases risk for dyslipidemia: a cohort study. Helicobacter. 2021;26:e12783.
33. Wang Z, Wang W, Gong R, Yao H, Fan M, Zeng J, et al. Eradication of *Helicobacter pylori* alleviates lipid metabolism deterioration: a large cohort propensity score-matched analysis. Lipids Health Dis. 2022;21:34–43.
34. Park J, Kim N, Kim WS, Lim SH, Choi Y, Jo HH, et al. Long-term effects of the eradication of *Helicobacter pylori* on metabolic parameters, depending on sex, in South Korea. Gut Liver. 2023;17:58–68.
35. Furuta T, Shirai N, Xiao F, Takashima M, Hanai H. Effect of *Helicobacter pylori* infection and its eradication on nutrition. Aliment Pharmacol Ther. 2002;16:799–806.
36. Fujiwara Y, Higuchi K, Arafa UA, Uchida T, Tominaga K, Watanabe T, et al. Long-term effect of *Helicobacter pylori* eradication on quality of life,

body mass index, and newly developed diseases in Japanese patients with peptic ulcer disease. Hepato-Gastroenterology. 2002;49:1298–302.

37. Kamada T, Hata J, Kusunoki H, Ito M, Tanaka S, Kawamura Y, et al. Eradication of *Helicobacter pylori* increases the incidence of hyperlipidaemia and obesity in peptic ulcer patients. Dig Liver Dis. 2005;37:39–43.
38. Murray LJ, Bamford KB, O'Reilly DP, McCrum EE, Evans AE. *Helicobacter pylori* infection: relation with cardiovascular risk factors, ischaemic heart disease, and social class. Br Heart J. 1995;74: 497–501.
39. Folsom AR, Nieto FJ, Sorlie P, Chambless LE, Graham DY. *Helicobacter pylori* seropositivity and coronary heart disease incidence. Atherosclerosis risk in communities (ARIC) study investigators. Circulation. 1998;98:845–50.
40. Pasceri V, Cammarota G, Patti G, Cuoco L, Gasbarrini A, Grillo RL, et al. Association of virulent *Helicobacter pylori* strains with ischemic heart disease. Circulation. 1998;97:1675–9.
41. Pellicano R, Mladenoval I, Broute N, Salmi LR, Mégraud F. Is there an association between *Helicobacter pylori* infection and coronary heart disease? Eur J Epidemiol. 1999;15:611–9.
42. Whincup P, Danesh J, Walker M, Lennon L, Thomson A, Appleby P. Prospective study of potentially virulent strains of *Helicobacter pylori* and coronary heart disease in middle-aged men. Circulation. 2000;101:1647–52.
43. Zhu J, Quyyumi AA, Muhlestein JB, Nieto FJ, Horne BD, Zalles-Ganley A, et al. Lack of association of *Helicobacter pylori* infection with coronary artery disease and frequency of acute myocardial infarction or death. Am J Cardiol. 2002;89:155–8.
44. Kinjo K, Sato H, Sato H, Shiotani I, Kurotobi T, Ohnishi Y, et al. Prevalence of *Helicobacter pylori* infection and its link to coronary risk factors in Japanese patients with acute myocardial infarction. Circ J. 2002;66:805–10.
45. Fraser AG, Scragg RK, Cox B, Jackson RT. Helicobacter pylori, chlamydia pneumoniae and myocardial infarction. Intern Med J. 2003;33:267–72.
46. Sheehan J, Kearney PM, Sullivan SO, Mongan C, Kelly E, Perry IJ. Acute coronary syndrome and chronic infection in the Cork coronary care case-control study. Heart. 2005;91:19–22.
47. Jin SW, Her SH, Lee JM, Yoon HJ, Moon SJ, Kim PJ, et al. The association between current *Helicobacter pylori* infection and coronary artery disease. Korean J Intern Med. 2007;22:152–6.
48. Zhang S, Guo Y, Ma Y, Teng Y. Cytotoxin-associated gene-A-seropositive virulent strains of *Helicobacter pylori* and atherosclerotic diseases: a systematic review. Chin Med J. 2008;121:946–51.
49. Tamer GS, Tengiz I, Ercan E, Duman C, Alioglu E, Turk UO. *Helicobacter pylori* seropositivity in patients with acute coronary syndromes. Dig Dis Sci. 2009;54:1253–6.
50. Khodaii Z, Vakili H, Ghaderian SM, Najar RA, Panah AS. Association of *Helicobacter pylori* infection with acute myocardial infarction. Coron Artery Dis. 2011;22:6–11.
51. Schöttker B, Adamu MA, Weck MN, Müller H, Brenner H. *Helicobacter pylori* infection, chronic atrophic gastritis and major cardiovascular events: a population-based cohort study. Atherosclerosis. 2012;220:569–74.
52. Lai CY, Yang TY, Lin CL, Kao CH. *Helicobacter pylori* infection and the risk of acute coronary syndrome: a nationwide retrospective cohort study. Eur J Clin Microbiol Infect Dis. 2015;34:69–74.
53. Liu J, Wang F, Shi S. *Helicobacter pylori* infection increase the risk of myocardial infarction: a meta-analysis of 26 studies involving more than 20,000 participants. Helicobacter. 2015;20:176–83.
54. Sun J, Rangan P, Bhat SS, Liu L. A meta-analysis of the association between *Helicobacter pylori* infection and risk of coronary heart disease from published prospective studies. Helicobacter. 2016;21:11–23.
55. Wang B, Yu M, Zhang R, Chen S, Xi Y, Duan G. A meta-analysis of the association between *Helicobacter pylori* infection and risk of atherosclerotic cardiovascular disease. Helicobacter. 2020;25:e12761.
56. Tong L, Wang B-B, Li F-H, Lv S-P, Pan F-F, Dong X-J. An updated meta-analysis of the relationship between *Helicobacter pylori* infection and the risk of coronary heart disease. Front Cardiovasc Med. 2022;9:794445.
57. Wang JW, Tseng KL, Hsu CN, Liang CM, Tai WC, Ku MK, et al. Association between *Helicobacter pylori* eradication and the risk of coronary heart diseases. PLoS One. 2018;13:e0190219.
58. Kim SB, Kim N, Park J, Hwang I-C, Lim SH, Song DH, et al. Preventive effect of *Helicobacter pylori* eradication on the coronary heart diseases depending on age and sex—a prospective study for up to 19 years. Helicobacter. 2023;28:e12969.
59. Mach F, Sukhova GK, Michetti M, Libby P, Michetti P. Influence of *Helicobacter pylori* infection during atherogenesis in vivo in mice. Circ Res. 2002;90:1–4.
60. Blum A, Tamir S, Mualem K, Ben-Shushan RS, Keinan-Boker L, Paritsky M. Endothelial dysfunction is reversible in *Helicobacter pylori*-positive subjects. Am J Med. 2011;124:1171–4.
61. Elizalde JI, Pérez-Pujol S, Heras M, Sionis A, Casanovas N, Martorell T, et al. Effects of *Helicobacter pylori* eradication on platelet activation and disease recurrence in patients with acute coronary syndromes. Helicobacter. 2004;9:681–9.
62. Stone AF, Mendall MA, Kaski JC, Edger TM, Risley P, Poloniecki J, et al. Effect of treatment for chlamydia pneumoniae and *Helicobacter pylori* on markers of inflammation and cardiac events in patients with acute coronary syndromes: South Thames trial of antibiotics in myocardial infarction and unstable angina (STAMINA). Circulation. 2002;106:1219–23.
63. Mendall MA, Patel P, Asante M, Ballam L, Morris J, Strachan DP, et al. Relation of serum cytokine

concentrations to cardiovascular risk factors and coronary heart disease. Heart. 1997;78:273–7.

64. Crabtree JE, Shallcross TM, Heatley RV, Wyatt JI. Mucosal tumour necrosis factor alpha and interleukin-6 in patients with *Helicobacter pylori* associated gastritis. Gut. 1991;32:1473–7.
65. Zhu J, Katz RJ, Quyyumi AA, Canos DA, Rott D, Csako G, et al. Association of serum antibodies to heat-shock protein 65 with coronary calcification levels: suggestion of pathogen-triggered autoimmunity in early atherosclerosis. Circulation. 2004;109:36–41.
66. Barton SG, Winrow VR, Rampton DS, Crabtree JE, Beales IL, Calam J. Circulating antibodies to the 60-kD heat shock protein (hsp) family in patients with *Helicobacter pylori* infection. Clin Exp Immunol. 1998;112:490–4.
67. Mayr M, Kiechl S, Mendall MA, Willeit J, Wick G, Xu Q. Increased risk of atherosclerosis is confined to CagA-positive *Helicobacter pylori* strains: prospective results from the Bruneck study. Stroke. 2003;34:610–5.
68. Kountouras J, Polyzos SA, Deretzi G, Katsinelos P, Kyriakou P. *Helicobacter pylori* infection and the risk for cardiovascular disease. Eur J Intern Med. 2011;22:e146–7.
69. Murray LJ, Bamford KB, Kee F, McMaster D, Cambien F, Dallongeville J, et al. Infection with virulent strains of *Helicobacter pylori* is not associated with ischaemic heart disease: evidence from a population-based case-control study of myocardial infarction. Atherosclerosis. 2000;149:379–85.
70. Meade TW, Mellows S, Brozovic M, Miller GJ, Chakrabarti RR, North WR, et al. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park heart study. Lancet. 1986;2:533–7.
71. Yarnell JW, Baker IA, Sweetnam PM, Bainton D, O'Brien JR, Whitehead PJ, et al. Fibrinogen, viscosity, and white blood cell count are major factors for ischaemic heart disease. The Caerphilly and speedwell collaborative heart disease studies. Circulation. 1991;83:836–44.
72. Patel P, Mendall MA, Carrington D, Strachan DP, Leatham E, Molineaux N, et al. Association of *Helicobacter pylori* and *chlamydia pneumoniae* infections with coronary heart disease and cardiovascular risk factors. Br Med J. 1995;311:711–4.
73. Fagoonee S, De Angelis C, Elia C, Silvano S, Oliaro E, Rizzetto M, et al. Potential link between *Helicobacter pylori* and ischemic heart disease: does the bacterium elicit thrombosis? Minerva Med. 2010;101:121–5.
74. Pellicano R, Oliaro E, Gandolfo N, Aruta E, Mangiardi L, Orzan F, et al. Ischemic cardiovascular disease and *Helicobacter pylori*. Where is the link? J Cardiovasc Surg. 2000;41:829–33.
75. Vijayvergiya R, Vadivelu R. Role of *Helicobacter pylori* infection in pathogenesis of atherosclerosis. World J Cardiol. 2015;7:134–43.
76. Lindsberg PJ, Grau AJ. Inflammation and infections as risk factors for ischemic stroke. Stroke. 2003;34:2518–32.
77. Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, et al. Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc Natl Acad Sci U S A. 1993;90:5791–5.
78. Franceschi F, Sepulveda AR, Gasbarrini A, Pola P, Silveri NG, Gasbarrini G, et al. Cross-reactivity of anti-CagA antibodies with vascular wall antigens: possible pathogenetic link between *Helicobacter pylori* infection and atherosclerosis. Circulation. 2002;106:430–4.
79. Niccoli G, Franceschi F, Cosentino N, Giupponi B, De Marco G, Merra G, et al. Coronary atherosclerotic burden in patients with infection by CagA-positive strains of *Helicobacter pylori*. Coron Artery Dis. 2010;21:217–21.
80. Pasceri V, Patti G, Cammarota G, Pristipino C, Richichi G, Di Sciascio G. Virulent strains of *Helicobacter pylori* and vascular diseases: a meta-analysis. Am Heart J. 2006;151:1215–22.
81. Franceschi F, Niccoli G, Ferrante G, Gasbarrini A, Baldi A, Candelli M, et al. CagA antigen of *Helicobacter pylori* and coronary instability: insight from a clinico-pathological study and a meta-analysis of 4241 cases. Atherosclerosis. 2009;202:535–42.
82. Moyaert H, Franceschi F, Roccarina D, Ducatelle R, Haesebrouck F, Gasbarrini A. Extragastric manifestations of *Helicobacter pylori* infection: other *Helicobacters*. Helicobacter. 2008;13 (Suppl 1):47–57.
83. Frustaci A, Chimenti C, Bellocci F, Morgante E, Russo MA, Maseri A. Histological substrate of atrial biopsies in patients with lone atrial fibrillation. Circulation. 1997;96:1180–4.
84. Bruins P, te Velthuis H, Yazdanbakhsh AP, Jansen PG, van Hardevelt FW, de Beaumont EM, et al. Activation of the complement system during and after cardiopulmonary bypass surgery: postsurgery activation involves C-reactive protein and is associated with postoperative arrhythmia. Circulation. 1997;96:3542–8.
85. Gaudino M, Andreotti F, Zamparelli R, Di Castelnuovo A, Nasso G, Burzotta F, et al. The -174G/C interleukin-6 polymorphism influences postoperative interleukin-6 levels and postoperative atrial fibrillation: is atrial fibrillation an inflammatory complication? Circulation. 2003;108 Suppl 1:II195–9.
86. Falk RH. Atrial fibrillation. N Engl J Med. 2001;344:1067–78.
87. Aviles RJ, Martin DO, Apperson-Hansen C, Houghtaling PL, Rautaharju P, Kronmal RA, et al. Inflammation as a risk factor for atrial fibrillation. Circulation. 2003;108:3006–10.
88. Chung MK, Martin OM, Sprecher D, Wazni O, Kanderian A, Carnes CA, et al. C-reactive protein elevation in patients with atrial arrhythmias: inflammatory mechanism and persistence of atrial fibrillation. Circulation. 2001;104:2886–91.
89. Montenero AS, Mollichelli N, Zumbo F, Antonelli A, Dolci A, Barberis M, et al. *Helicobacter pylori* and

atrial fibrillation: a possible pathogenic link. Heart. 2005;91:960–1.

90. Andrew P, Montenero AS. Is *Helicobacter pylori* a cause of atrial fibrillation? Futur Cardiol. 2006;2:429–39.
91. Badran HM, Mahfouz ME. Cytotoxin-associated gene-a bearing strains of *Helicobacter pylori* and atrial fibrillation due to ischemic origin: is there a link? Eur J Cardiovasc Prev Rehabil. 2007;14:518–20.
92. Ki MR, Shin DG, Park JS, Hong KS, Hong IH, Park JK, et al. Frequency of vacuolating cytotoxin a (VacA)-positive *Helicobacter pylori* seropositivity and TGF-β1 decrease in atrial fibrillation. Int J Cardiol. 2010;145:345–6.
93. Bunch TJ, Day JD, Anderson JL, Horne BD, Muhlestein JB, Crandall BG, et al. Frequency of *Helicobacter pylori* seropositivity and C-reactive protein increase in atrial fibrillation in patients undergoing coronary angiography. Am J Cardiol. 2008;101:848–51.
94. Platonov P, Ekesbo R, Hasson A, Andsberg E, Meurling CJ, Nilsson I, et al. Permanent atrial fibrillation in patients without structural heart disease is not associated with signs of infection by chlamydia pneumoniae and *Helicobacter pylori*. Acta Cardiol. 2008;63:479–84.
95. Lunetta M, Fazio G, Avena V, Corrado E, Loredana S, Arnone E, et al. *Helicobacter pylori* and atrial fibrillation: absence of correlations. J Cardiovasc Med. 2009;10:4–5.
96. Franceschi F, Brisinda D, Buccelleetti F, Ruggieri MP, Gasbarrini A, Sorbo A, et al. Prevalence of virulent *Helicobacter pylori* strains in patients affected by idiopathic dysrhythmias. Intern Emerg Med. 2013;8:333–7.
97. Wang D-Z, Chen W, Yang S, Wang J, Li Q, Fu Q, et al. *Helicobacter pylori* infection in Chinese patients with atrial fibrillation. Clin Interv Aging. 2015;10:813–9.
98. Yan J, She Q, Zhang Y, Cui C, Zhang G. The association between arrhythmia and *Helicobacter pylori* infection: a meta-analysis of case–control studies. Int J Environ Res Public Health. 2016;13:1139.
99. Tetta C, Moula AI, Matteucci F, Parise O, Maesen B, Johnson D, et al. Association between atrial fibrillation and *Helicobacter pylori*. Clin Res Cardiol. 2019;108:730–40.

35

Extraintestinal Manifestations of *H. pylori* Infection: Asthma and Allergic Disorders

Cheol Min Shin

Abstract

Helicobacter pylori (*H. pylori*) prevalence decreases, while asthma and allergic disorders rapidly increase mainly in Western developed countries. Numerous epidemiological surveys have reported that there is a meaningful trade-off relationship between *H. pylori* infection and allergic diseases. Allergic asthma is induced by T cells that secrete Th2 cytokines, such as interleukin (IL)-4 and IL-5. *H. pylori* infection is thought to not only reinforce Th1 immune responses but also suppress Th2 reactions. According to recent studies using animal models of allergic airway disease, *H. pylori* is expected to reduce airway hypersensitivity by directly inducing regulatory T-cell expressions via dendritic cells. As *H. pylori* prevalence tends to decrease in East Asian countries such as Korea and Japan, mainly among children and adolescents, allergic diseases such as atopic dermatitis, asthma, and allergic rhinitis rapidly increase. Thus, epidemiological surveys on these populations regarding the correlation are warranted in the future.

Keywords

Helicobacter pylori · Asthma · Allergic disorders

35.1 Introduction

Helicobacter pylori (*H. pylori*) infection has been acknowledged as the main cause of upper gastrointestinal tract disorders, such as gastritis, peptic ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. However, there have been several lines of studies, which are mainly from the United States and European countries and have reported the association between the reduction of *H. pylori* infection and the increment of asthma and allergic disorders. This trade-off relationship between exposure to external allergens or microorganisms and asthma is widely acknowledged, which has been explained by the hygiene hypothesis that is now called as disappearing microbiota hypothesis. This chapter is going to introduce previous epidemiological research studies on the relationship between *H. pylori* and asthma, the pathophysiology of allergic disorders, and the theoretical evidence of the causality on *H. pylori* infection, asthma, and allergic diseases [1].

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35.2 *H. pylori* and Allergic Asthma: Epidemiological Evidence

H. pylori has been coexisted with mankind throughout human history. *H. pylori* initially colonizes itself inside the human stomach and then induces chronic immunologic and inflammatory reactions [2, 3]. However, *H. pylori* infection during the juvenile period has rapidly decreased during the process of industrialization, especially in the United States and European countries [4]. The causes of this reduction are thought to be improvements on hygiene, reduced family-oriented infection due to the decreased number of family members, supplying clean water, and the disappearance of feeding children with chewed foods from their mother or grandmother. Particularly, the broad use of antibiotics among pediatrics is thought to be one important reason for lowering *H. pylori* prevalence in childhood [5].

The associations between *H. pylori* infection and asthma and allergic disorders have been reported in cross-sectional studies [6–8]. According to the study on the adults of Sweden, Iceland, and Estonia, there was a meaningful correlation between positive serum anti-*H. pylori* antibody and the prevalence of asthma and hay fever [9]. In addition, there were meaningful trade-off relationships among the prevalence of *H. pylori*, asthma, and allergy, based on the results from the National Health and Nutrition Examination III (NHANES III) study [10]. In particular, *CagA*-positive strains showed a stronger relationship with allergy and asthma. Nonetheless, there have also been numerous studies that showed no or minimal correlations between *H. pylori* infection and asthma [11–15]. Notably, the significant relationship between *H. pylori* infection and asthma is often observed in young and pediatric asthmatics. According to the subgroup analyses that utilized the NHANES III cohort, there was a strong relationship with the population less than 43 years old (odds ratio [OR] 0.63; 95% confidence interval [CI], 0.43–0.93). However, the association in the population over 43 years old was statistically insignificant. Also, there was a statistically significant relationship in those with asthma diagnosed before the age of 15 (OR 0.63; 95% CI, 0.43–0.93), while the relationship was insignificant among patients who were initially diagnosed with asthma when they became adults. Follow-up studies showed similar results on pediatric asthma [16, 17]. A recent longitudinal study showed a negative correlation between asthma with wheezing sounds and positive serum anti-*H. pylori* antibodies, but there was no significant association between asthma, allergic rhinitis, and atopic dermatitis [18]. Moreover, the recent meta-analyses of previous studies suggested that *H. pylori* infection has a weak negative correlation with asthma, especially more in pediatrics than in adults [19, 20].

Asthma diagnosed in the young may be different in terms of etiological aspects from that diagnosed initially during adulthood. Pediatric asthma is often accompanied by atopic dermatitis and allergic rhinitis (allergic march) and is related to primed allergen, IgE mediation, and Th2 inflammation. In comparison, adult asthma originates from various causes, such as smoking, air pollution, and certain jobs, other than allergen. Also, patients defined as "adult asthma" are heterogeneous because they include those with pediatric asthma continued to adulthood and those with recurrent asthma symptoms since childhood, as well as those with asthma initially diagnosed when they were adults. In addition, the interpretation of the previous studies requires caution. Several studies with insignificant results have limitations related to the small sample size to obtain statistical significance, and there can be a problem of misclassification because of diagnosing asthma based on its wheezing sound and the difficulty of infant and pediatric asthma, from the methodological perspective [21]. Moreover, most epidemiological studies used serum *H. pylori* antigen test to confirm *H. pylori* infection, but the serologic test cannot differentiate past infection from the active one, and some patients with remote past infection are apt to be classified as negative infection (false negatives) due to their decreased serum antibody titer [22].

35.3 *H. pylori* and Allergic Asthma: Proposed Mechanisms Underlying Protective Effects of *H. pylori*

Evidence based on epidemiological studies has limitation itself, as it shows the association between *H. pylori* infection and allergic asthma only and cannot prove a causal relationship between these two factors. Previously, there have been only a few basic studies on the mechanism of how *H. pylori* infection protects asthma. However, recent studies might suggest several possible mechanisms.

35.3.1 Basic Concept on the Pathophysiology of Allergic Asthma

Symptoms of asthma originate from airway hyperresponsiveness (AHR) and reversible airway obstruction attributed to chronic allergic inflammation of airway, which leads to recurrent episodes of breathlessness, cough, chest tightness, and wheezing. However, the pathophysiology of these symptoms is complex. For example, cellular assessment of sputum can classify the patients into either eosinophil-predominant or neutrophil-predominant or mixed inflammation. Patients with eosinophilic asthma show a better response to treatment with inhaled corticosteroids compared with those with neutrophil-predominant inflammation. Therefore, although there is no explicit immune-pathological difference based on the clinical symptoms, patients with more eosinophils on their sputum examinations show better responsiveness and prognosis on inhaled corticosteroids than those who have more neutrophils on their sputum [23]. In terms of genetic susceptibility, “Th2-high” patients, who exhibit increased expression of Th2 cytokine (i.e., interleukin (IL)-4 and IL-13) response genes such as periostin, show a better response on inhaled corticosteroid therapy than “Th2-low” patients, who do not have those genetic expressions [24].

Recently, animal models of allergic airway disease have contributed to a better understanding of the mechanisms underlying allergic asthma. It has been identified that Th2 cells and Th2 cytokines, such as IL-4, IL-5, and IL-13, are important for allergen-induced inflammation and AHR [6], and the contribution of secreted IL-17 by Th17 cells on AHR, via direct stimulation of airway smooth muscles, has been identified [25]. Once the epithelial cells in the respiratory tract are exposed to a specific allergen, they are activated and release epithelial cytokines including IL-25, IL-33, and thymic stromal lymphopoietin that involve the activation of dendritic cells (DCs) and the movement to the regional lymph nodes. DCs are believed to induce the sensitization of certain inhaled allergens and recruit inflammatory cells [26]. In particular, IL-33 can activate mast cell and basophil, which works with IL-25 to activate innate lymphoid cells (ILCs). Activated ILCs secrete IL-5 and IL-13, which are known as Th2 cytokines, and participate in the induction of bronchial allergic inflammatory reaction. During the sensitization process, IL-4 increases the Th2 cell ratio in regional lymph nodes, and the Th2 cells secrete IL-4, IL-5, and IL-13. Among them, IL-5 is important in the survival of eosinophil, IL-13 directly works on the epithelial cells to secrete mucin and chemokines, and IL-13 also works on the activation of smooth muscle macrophages to induce AHR [27, 28].

35.3.2 Hygiene Hypothesis and Disappearing Microbiota Hypothesis

The recent increment of patients with allergic disorders in developed countries cannot be explained simply by genetic factors; socioeconomic factors such as nutrition and hygiene, air pollution, and environmental exposure to tobacco smoking or microbial components are thought to be more important in the development of asthma [6]. Hygiene hypothesis is the most popular one to explain this phenomenon. The hypothesis explains that the reduced frequency and severity of encountering microbial antigens via food, air, soil, or water have led to an increase in allergic disorders [29]. There have been several studies that suggest trade-off relationships between asthma and type A hepatitis, *Toxoplasma gondii* infection, and herpes simplex virus type 1 infection [30], and one study has shown the negative correlation of asthma with residence in rural areas among children in southern Germany, because residents in a rural area are thought to be exposed more frequently to the various microorganisms [31]. However, the hygiene hypothesis makes sense to explain only pediatric asthma and atopic disorders. Therefore, there is a limitation to apply this hypothesis to adult asthma that multiple factors are related as mentioned above.

However, the disappearing microbiota hypothesis explains that a fetus is germ-free in utero but becomes exposed postpartum to various microorganisms that inhabit exposed mucosal surfaces such as the skin, nasal and oral cavity, vagina, and gut, in which these microflora constitute a microbiota. The microbiota is considered to play important roles in the immune system after birth [32]. In particular, the gut microbiota could be affected by host factors and environmental factors such as labor method and breastfeeding from the earliest life. To date, numerous animal and clinical studies have reported the correlation between the gut microbiota and atopic disorders [33].

It is still unclear how the intestinal microbiota affects the immune system, but the commensal microbiota might regulate the balance of Th1/Th2 immune responses. Th2 reaction is reinforced when there is no microbial colony, whereas microbial antigen in the intestinal tract is expected to stimulate Th1 reaction to generate protective effects on atopic disorders and asthma by Th2 cells [34]. Also, gut microbiota induces T cells to differentiate into regulatory T cells (Treg), which secrete cytokines such as IL-10 and transforming growth factor (TGF)-β to induce anti-inflammatory reactions [32]. DCs on intestinal mucosa deliver antigens to naïve T cells, and they induce T cells to differentiate into the effect T cell or Treg. The DCs may selectively control the action of Toll-like receptor (TLR), so unnecessary inflammatory reaction toward indigenous microorganism does not occur to maintain immunologic tolerance. Both DCs and macrophages secrete IL-10, TGF-β, and retinoic acid, which induces the differentiation of the undifferentiated T cells into $CD4^{+}FoxP3^{+}$Treg [35]. According to a recent study, increased sensitivity on inflammatory bowel disease and allergic respiratory disorders was reported in germ-free mice attributed to the loss of variety in natural killer T cells on lung and intestinal mucosal membranes [36]. In addition, this hypothesis was supported by a flora change after antibiotic treatment that induced the increase in serum IgE concentration and the increase in circulating basophils that led to an exaggerated basophil-mediated Th2 cell reaction and allergic inflammatory reaction [37].

35.3.3 Theoretical Hypothesis to Explain Protective Effect by *H. pylori*

Allergic asthma is induced by T cells that secrete Th2 cytokine and suppress Th1 reaction, and *H. pylori* is thought to stimulate Th1 immune responses to protect against allergic disorders. Several studies reported that *H. pylori* neutrophil-activating protein (HP-NAP) played an important regulatory role, while *H. pylori* infection stimulated Th1 mucosal immune responses [38–40]. According to these studies, HP-NAP repressed the accumulation of eosinophils and the increase of serum IgE proliferation in alveoli when asthma is induced by ovalbumin in allergic asthma animal model [38]. These findings suggest that HP-NAP could be an important substance to elucidate asthma protection by *H. pylori* [39, 41]. From this viewpoint, recent studies have reported that HP-NAP may be a potential therapeutic agent in the prevention and treatment of allergic asthma and atopic dermatitis [42, 43].

Moreover, airway hypersensitivity was remarkably reduced among *H. pylori*-infected mice; when both newborn and adult mice were infected with *H. pylori* and then sensitized with ovalbumin in the allergic airway disease mouse model, eosinophil infiltration and IL-5 and IL-13 secretions were substantially reduced. In addition, effector T cells that secrete IL-5 and IL-17A in the lungs were also reduced [44]. Interestingly, these effects were dominant in neonatally infected mice, while respiratory tract infection and airway hypersensitivity reduction were minimal among adult-infected mice. According to one study, *H. pylori* directly works on DCs in the gastric mucosae to induce immunologic tolerance, by which allergy-inducing T-cell activation is suppressed [45]. That is, DCs that are exposed to *H. pylori* move to gut-draining mesenteric lymph nodes and induce TGF-β-dependent FoxP3^{+}Treg differentiation, and Treg inhibits *H. pylori*-specific Th1/Th7 immune response and excessive inflammatory reaction by *H. pylori* infection on gastric mucosa. Also, Treg is transported to the lungs via the bloodstream to induce immunologic tolerance of lung DCs, and Treg secretes cytokines such as IL-10 to suppress Th2/Th17 response by certain allergens that are important for the pathophysiology of allergic asthma [46] (Fig. 35.1). These procedures are unrelated to *H. pylori* toxic factors, such as VacA and CagA, and seem to be rather related to cytokines such as TGF-β and IL-10 [47].

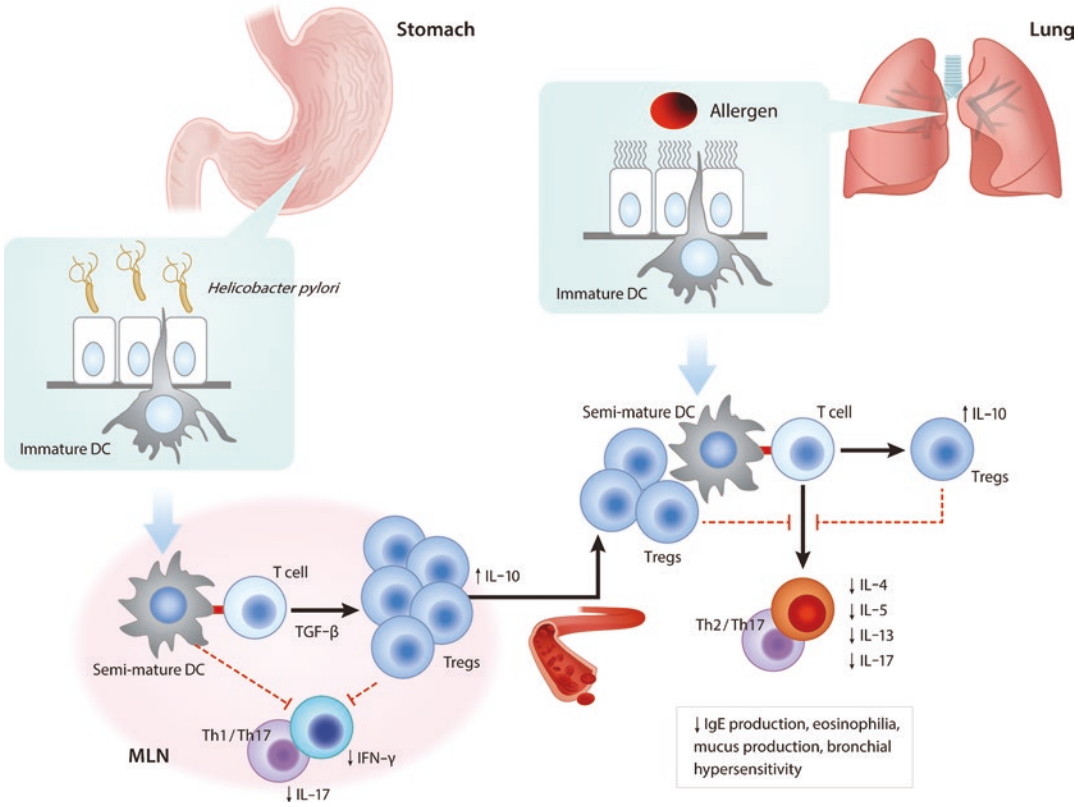
Stomach
Helicobacter pylori
Immature DC
Semi-mature DC
T cell
TGF-β
↑IL-10
Tregs
Th1/Th17
↓IFN-γ
↓IL-17
MLN
Lung
Allergen
Immature DC
Semi-mature DC
T cell
↑IL-10
Tregs
Tregs
Th2/Th17
↓IL-4
↓IL-5
↓IL-13
↓IL-17
↓IgE production, eosinophilia,
mucus production, bronchial
hypersensitivity

Fig. 35.1 Underlying mechanism of airway hyperresponsiveness suppressed by *H. pylori* infection. (1) Immature dendritic cells (DCs) can be activated by *H. pylori*. Then, the semi-mature DCs move to gut-draining mesenteric lymph nodes (MLNs), (2) where they induce the expression of regulatory T cells (*Treg*). (3) Tregs move to the lungs via bloodstream (4) to induce immunologic tolerance of lung (DCs) or (5) to directly suppress the Th2 reaction. (6) It leads to the decrease in IgE production/eosinophilia. (Modified from Taube and Müller [6], and Arnold et al. [46])

35.4 Conclusions

The trade-off relationship among *H. pylori* infection, asthma, and allergy is based on epidemiological surveys in the United States and European countries, where *H. pylori* prevalence has rapidly decreased in these regions for the past several decades. The correlation between *H. pylori* infection and asthma is more prominent in pediatric asthma because pediatric asthma and adult asthma are thought to be different in a pathophysiologic aspect. Recently, theoretical evidence suggested that *H. pylori* infection alleviates airway hypersensitivity by allergens in an immunological aspect, based on allergic airway disease animal model. *H. pylori* prevalence tends to decrease in East Asian countries such as Korea and Japan, mainly among young children and adolescents, and allergic diseases such as atopic dermatitis, asthma, and allergic rhinitis rapidly increase. So, epidemiologic surveys on these populations are warranted regarding this issue in the future.

References

1. Shin CM. Extra-intestinal manifestation of *Helicobacter pylori* infection: allergic diseases including asthma, atopy and idiopathic urticaria. Korean J Med. 2013;84:774–80.
2. Thomas JE, Dale A, Harding M, Coward WA, Cole TJ, Weaver LT. *Helicobacter pylori* colonization in early life. Pediatr Res. 1999;45:218–23.
3. Rothenbacher D, Inceoglu J, Bode G, Brenner H. Acquisition of *Helicobacter pylori* infection in a high-risk population occurs within the first 2 years of life. J Pediatr. 2000;136:744–8.
4. Eder W, Ege MJ, von Mutius E. The asthma epidemic. N Engl J Med. 2006;355:2226–35.
5. Blaser MJ, Chen Y, Reibman J. Does *Helicobacter pylori* protect against asthma and allergy? Gut. 2008;57:561–7.
6. Taube C, Müller A. The role of *Helicobacter pylori* infection in the development of allergic asthma. Expert Rev Respir Med. 2012;6:441–9.
7. Kim JH, Kim N, Lim SH, Kwon JW, Shin CM, Chang YS, et al. Inverse relationship between *Helicobacter pylori* infection and asthma among adults younger than 40 years: a cross-sectional study. Medicine (Baltimore). 2016;95:e2609.
8. Wang D, Chen Y, Ding Y, Tu J. Inverse association between *Helicobacter pylori* infection and childhood asthma in a physical examination population: a cross-sectional study in Chongqing, China. BMC Pediatr. 2022;22:615.
9. Thjodleifsson B, Asbjörnsdottir H, Sigurjonsdottir RB, Gíslason D, Olafsson I, Cook E, et al. Seroprevalence of *Helicobacter pylori* and cagA antibodies in Iceland, Estonia and Sweden. Scand J Infect Dis. 2007;39:683–9.
10. Chen Y, Blaser MJ. Inverse associations of *Helicobacter pylori* with asthma and allergy. Arch Intern Med. 2007;167:821–7.
11. Matricardi PM, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M, et al. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. BMJ. 2000;320:412–7.
12. Tsang KW, Lam WK, Chan KN, Hu W, Wu A, Kwok E, et al. *Helicobacter pylori* sero-prevalence in asthma. Respir Med. 2000;94:756–9.
13. Jarvis D, Luczynska C, Chinn S, Burney P. The association of hepatitis a and *Helicobacter pylori* with sensitization to common allergens, asthma and hay fever in a population of young British adults. Allergy. 2004;59:1063–7.
14. Jun ZJ, Lei Y, Shimizu Y, Dobashi K, Mori M. *Helicobacter pylori* seroprevalence in patients with mild asthma. Tohoku J Exp Med. 2005;207:287–91.
15. Pessi T, Virta M, Adjers K, Karjalainen J, Rautelin H, Kosunen TU, et al. Genetic and environmental factors in the immunopathogenesis of atopy: interaction of *Helicobacter pylori* infection and IL4 genetics. Int Arch Allergy Immunol. 2005;137:282–8.
16. Chen Y, Blaser MJ. *Helicobacter pylori* colonization is inversely associated with childhood asthma. J Infect Dis. 2008;198:553–60.
17. Amberbir A, Medhin G, Erku W, Alem A, Simms R, Robinson K, et al. Effects of *Helicobacter pylori*, geohelminth infection and selected commensal bacteria on the risk of allergic disease and sensitization in 3-year-old Ethiopian children. Clin Exp Allergy. 2011;41:1422–30.
18. Zevit N, Balicer RD, Cohen HA, Karsh D, Niv Y, Shamir R. Inverse association between *Helicobacter pylori* and pediatric asthma in a high-prevalence population. Helicobacter. 2012;17:30–5.
19. Zhou X, Wu J, Zhang G. Association between *Helicobacter pylori* and asthma: a meta-analysis. Eur J Gastroenterol Hepatol. 2013;25:460–8.
20. Wang Q, Yu C, Sun Y. The association between asthma and *Helicobacter pylori*: a meta-analysis. Helicobacter. 2013;18:41–53.
21. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med. 1995;332:133–8.

22. Lee JH, Kim N, Chung JI, Kang KP, Lee SH, Park YS, et al. Long-term follow up of *Helicobacter pylori* IgG serology after eradication and reinfection rate of *H. pylori* in South Korea. Helicobacter. 2008;13:288–94.
23. Haldar P, Pavord ID. Noneosinophilic asthma: a distinct clinical and pathologic phenotype. J Allergy Clin Immunol. 2007;119:1043–52.
24. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. Am J Respir Crit Care Med. 2009;180:388–95.
25. Kudo M, Melton AC, Chen C, Engler MB, Huang KE, Ren X, et al. IL-17A produced by alphabeta T cells drives airway hyper-responsiveness in mice and enhances mouse and human airway smooth muscle contraction. Nat Med. 2012;18:547–54.
26. Lambrecht BN, Hammad H. The role of dendritic and epithelial cells as master regulators of allergic airway inflammation. Lancet. 2010;376:835–43.
27. Taube C, Duez C, Cui ZH, Takeda K, Rha YH, Park JW, et al. The role of IL-13 in established allergic airway disease. J Immunol. 2002;169:6482–9.
28. Locksley RM. Asthma and allergic inflammation. Cell. 2010;140:777–83.
29. Blaser MJ, Falkow S. What are the consequences of the disappearing human microbiota? Nat Rev Microbiol. 2009;7:887–94.
30. Matricardi PM, Rosmini F, Panetta V, Ferrigno L, Bonini S. Hay fever and asthma in relation to markers of infection in the United States. J Allergy Clin Immunol. 2002;110:381–7.
31. Braun-Fahrländer C, Riedler J, Herz U, Eder W, Waser M, Grize L, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med. 2002;347:869–77.
32. McLoughlin RM, Mills KH. Influence of gastrointestinal commensal bacteria on the immune responses that mediate allergy and asthma. J Allergy Clin Immunol. 2011;127:1097–107.
33. Ly NP, Litonjua A, Gold DR, Celedón JC. Gut microbiota, probiotics, and vitamin D: interrelated exposures influencing allergy, asthma, and obesity? J Allergy Clin Immunol. 2011;127:1087–94.
34. Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. J Immunol. 1998;160:4730–7.
35. Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. Nat Immunol. 2007;8:1086–94.
36. Olszak T, An D, Zeissig S, Vera MP, Richter J, Franke A, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. Science. 2012;336:489–93.
37. Hill DA, Siracusa MC, Abt MC, Kim BS, Kobuley D, Kubo M, et al. Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. Nat Med. 2012;18:538–46.
38. Codolo G, Mazzi P, Amedei A, Del Prete G, Berton G, D'Elios MM, et al. The neutrophil-activating protein of *Helicobacter pylori* down-modulates Th2 inflammation in ovalbumin-induced allergic asthma. Cell Microbiol. 2008;10:2355–63.
39. D'Elios MM, Codolo G, Amedei A, Mazzi P, Berton G, Zanotti G, et al. *Helicobacter pylori*, asthma and allergy. FEMS Immunol Med Microbiol. 2009;56:1–8.
40. Zhou S, Huang Y, Liang B, Dong H, Yao S, Chen Y, et al. Systemic and mucosal pre-administration of recombinant *Helicobacter pylori* neutrophil-activating protein prevents ovalbumin-induced allergic asthma in mice. FEMS Microbiol Lett. 2017;364:fnw288.
41. Fu HW, Lai YC. The role of *Helicobacter pylori* neutrophil-activating protein in the pathogenesis of *H. pylori* and beyond: from a virulence factor to therapeutic targets and therapeutic agents. Int J Mol Sci. 2023;24:91.
42. Guo X, Ding C, Lu J, Zhou T, Liang T, Ji Z, et al. HP-NAP ameliorates OXA-induced atopic dermatitis symptoms in mice. Immunopharmacol Immunotoxicol. 2020;42:416–22.
43. Mai J, Liang B, Xiong Z, Ai X, Gao F, Long Y, et al. Oral administration of recombinant *Bacillus subtilis* spores expressing *Helicobacter pylori* neutrophil-activating protein suppresses peanut allergy via up-regulation of Tregs. Clin Exp Allergy. 2019;49:1605–14.
44. Arnold IC, Dehzad N, Reuter S, Martin H, Becher B, Taube C, et al. *Helicobacter pylori* infection prevents allergic asthma in mouse models through the induction of regulatory T cells. J Clin Invest. 2011;121:3088–93.
45. Oertli M, Sundquist M, Hitzler I, Engler DB, Arnold IC, Reuter S, et al. DC-derived IL-18 drives Treg differentiation, murine *Helicobacter pylori*-specific immune tolerance, and asthma protection. J Clin Invest. 2012;122:1082–96.
46. Arnold IC, Hitzler I, Müller A. The immunomodulatory properties of *Helicobacter pylori* confer protection against allergic and chronic inflammatory disorders. Front Cell Infect Microbiol. 2012;2:10.
47. Kao JY, Zhang M, Miller MJ, Mills JC, Wang B, Liu M, et al. *Helicobacter pylori* immune escape is mediated by dendritic cell-induced Treg skewing and Th17 suppression in mice. Gastroenterology. 2010;138:1046–54.

Extragastric Manifestations of *H. pylori* Infection: Lower GI Disorders

36

Jae Yong Park

Abstract

Possible association has been suggested between *Helicobacter pylori* (*H. pylori*) infection and some of the lower gastrointestinal (GI) tract diseases. Previous studies have demonstrated that *H. pylori* infection might be associated with an increased risk of colorectal cancer/adenoma, while it seems to harbor a protective role in the development of inflammatory bowel disease. Some of them suggest possible differences in the association according to race/ethnicity. There are also a few studies on the effect of *H. pylori* eradication treatment on the development or aggravation of these diseases. Plausible mechanisms include both direct and indirect effects of *H. pylori*, such as influencing the host immune response and changing the intestinal microbiota, leading to the pathologic condition of the gut. However, the majority of the studies on this subject have the limitation of being simple epidemiologic studies, and definite proof for causal relationship or exact underlying mechanism has not been clearly demonstrated in most conditions yet. In this chapter, we will review the previous literature on the association between *H. pylori* infection and some lower GI tract diseases and discuss possible underlying mechanisms.

Keywords

Helicobacter pylori · Lower gastrointestinal tract disease · Colorectal cancer · Inflammatory bowel disease · Irritable bowel syndrome

36.1 Introduction

It is well-known that there is a strong association between various upper gastrointestinal (GI) tract diseases and *Helicobacter pylori* (*H. pylori*) infection. Nowadays, there have been some studies suggesting possible correlation between *H. pylori* infection with extra-gastric, or even extra-GI diseases. This association has been suggested in a few conditions of lower GI tract, including colorectal cancer (CRC)/adenoma, inflammatory bowel disease (IBD), or irritable bowel syndrome (IBS).

Although this is rather an interesting topic, there is a limitation that the association is mostly inferred based on simple cross-sectional or epidemiologic studies. Indeed, definite proof for causal relationship or exact underlying mechanism has not been clearly demonstrated in most of the conditions yet. There have been attempts to explain the relationship between *H. pylori* infection and lower GI disorders by changes in the gut microbiome or immune modulation. In this

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chapter, we will address clinical and experimental evidences and discuss the current perspectives on the association between lower GI tract disorders and *H. pylori* infection.

36.2 Colorectal Cancer or Adenoma

36.2.1 Clinical Studies

Many studies have been conducted on the association between *H. pylori* and CRC. Numerous reports, mainly cross-sectional epidemiologic studies, and recent meta-analysis studies suggest that there is a positive correlation between the prevalences of *H. pylori* infection and CRC/adenomas [1–4].

Four recent meta-analyses, which included 16 countries in Asia, Europe, America, Middle East, and Oceania, demonstrated a positive correlation between *H. pylori* infection and the risk of colorectal neoplasia (Table 36.1). The odd ratio (OR, 95% confidence interval [CI]) ranged from 1.27 (1.17–1.37) to 1.44 (1.26–1.65) for CRC and 1.49 (1.37–1.62) to 1.66 (1.39–1.97) for colorectal adenoma [1].

In a Korean cohort study comprising 4466 subjects who underwent screening upper and lower GI endoscopy, the overall *H. pylori* infection rate was greater in adenoma cases (1245) than in controls without polyps (3221) (55.0 vs. 48.5%, p < 0.001) [2]. *H. pylori* infection was still associated with an increased risk of colorectal adenomas after adjusting for various other risk factors (OR 1.28; 95% CI, 1.11–1.47). In particular, subgroup analyses showed that this tendency was more prominent in the advanced adenoma group (OR 1.84; 95% CI, 1.25–2.70) or multiple (≥3) adenoma group (OR 1.72; 95% CI, 1.26–2.35). Regarding the location of adenomas, this association was not evident when the adenomas were only limited in the rectum (OR 0.85; 95% CI, 0.58–1.24).

An Asian cross-sectional study with 6351 asymptomatic subjects analyzed the relationship between the presence of serum anti-*H. pylori* IgG antibodies and advanced colorectal neoplasm detected on a screening lower GI endoscopy [3]. In logistic regression multivariate analysis, *H. pylori* infection was an independent risk factor of overall and advanced colorectal neoplasm (OR 1.34; 95% CI, 1.04–1.72). Interestingly, when the presence of atrophic gastritis was also considered as well as *H. pylori* infection, *H. pylori*-related atrophic gastritis was significantly associated with the risk of advanced colorectal neoplasm (OR 1.40; 95% CI, 1.03–1.91; p = 0.030), while *H. pylori* infection without atrophic gastritis or atrophic gastritis without *H. pylori* infection was not. Considering that atrophic gastritis occurs as a result of chronic *H. pylori* infection, it could be inferred that long-term *H. pylori* infection with subsequent physiologic changes may be involved in the development of CRC or precancerous lesions.

There was also a study investigating the association of seropositivity of *H. pylori* proteins

Table 36.1 Studies included in the recent meta-analyses on the association between *H. pylori* and colorectal cancer and adenoma

Meta-analysis (year)	Number of included studies	Included countries	Colorectal cancer OR (95% CI)	Colorectal adenoma OR (95% CI)
Wu et al. (2013) [5]	27	Europe (11), USA (4), East Asia (8), Middle East (3), and Brazil (1)	1.39 (1.18–1.64)	1.66 (1.39–1.97)
Zhao et al. (2016) [6]	14	Europe (6), USA (3), East Asia (3), Middle East (1), and Australia (1)	1.33 (1.01–1.77)	
Yang et al. (2019) [7]	27	–	1.27 (1.17–1.37)	
Choi et al. (2020) [8]	48	Europe (14), USA (9), East Asia (16), Middle East (6), Thailand (1), Australia (1), and Brazil (1)	1.44 (1.26–1.65)	1.49 (1.37–1.62)

with risk of CRC, in a large prospective US cohort consortium including various ethnic groups of people [4]. Overall, *H. pylori* seropositivity failed to show a significant increase in the odds of CRC in either all subjects (OR 1.09; 95% CI, 0.99–1.20) or in each ethnic subgroup. However, seropositivity to 4 individual *H. pylori* proteins (VacA, GroEL, Omp, and HcpC) was significantly associated with a 10–11% increased odds of CRC. The strength of the association was variable between different races/ethnicities, with African Americans having the strongest association of *H. pylori* + VacA+ with odds of CRC (OR 1.45; 95% CI, 1.08–1.96). Furthermore, the odds of CRC were significantly correlated with strong antibody responses to the VacA. Differences according to race/ethnicity might be attributed to the diversity of VacA subtypes and variable host immune responses; activated VacA appears higher in African Americans or Asian Americans than in Caucasians.

One of the strong evidences for a causal relationship is to see if the risk of disease decreases when the risk factor is removed. There have been some studies conducted to investigate the effect of *H. pylori* eradication therapy on the risk of CRC or adenoma. A retrospective cohort study in Taiwan followed 615 patients for 9 years with no previous history of CRC or adenoma [9]. During follow-up, the incidence rates of colorectal adenoma in participants with persistent *H. pylori* infections and those with successful eradication were 160.5 and 51.6 per 1000 person-years, respectively (p = 0003). The multivariate logistic regression showed that the hazard ratio (HR) for adenoma was significantly higher in patients with persistent *H. pylori* infection than successful eradication group (HR 3.04; 95% CI, 1.75–5.28).

36.2.2 Possible Mechanisms for Causal Effects

Although the causal relationship has not been clearly proven and there are very limited studies on the potential mechanisms for causal effects of *H. pylori* on CRC, several potential mechanisms have been proposed [10] (Fig. 36.1). The question is how this unique strain might directly or indirectly affect the development of colorectal neoplasm, far from its main habitat.

The presence of the bacterium or molecular effectors, such as produced toxins, in the relevant colorectal tissue would constitute a direct effect. Although scarce, there are studies which demonstrated the presence of *H. pylori* in colorectal tumor tissue. *H. pylori* or its DNA has been identified in polyps or CRC fragments, by IHC or PCR [11–13]. Adhesins can function as biological effector molecules when bacteria adhere to a cell, causing a series of events. Indeed, ability of some virulence factors of *H. pylori* to exert their effects outside the stomach has previously been shown in animal colitis models [14]. If *H. pylori* can directly exhibit carcinogenic effect to the colorectal epithelial cells, it would be through virulence factors such as VacA. They might exhibit carcinogenic effects in the colonic epithelium, including cellular vacuolation, disruption of cellular pathways, changes in epithelial permeability, and immunomodulatory and proinflammatory effects [15]. However, the major limitation of this hypothesis is that *H. pylori* only infects the gastric type of mucosa and has never been observed adhering to the colonic epithelium in vivo.

There are also several hypotheses regarding how *H. pylori* could indirectly contribute to the development of CRC. First, *H. pylori* infection may alter the microbial niche of the gut, which may then influence the development of CRC. Gastric mucosal atrophy secondary to chronic *H. pylori* infection leads to reduced acid secretion, subsequently causing alterations in the population and profiles of colorectal microbiota [16]. Previous studies have shown that particular bacteria or bacterial dysbiosis in the gut can promote the development or progression of CRC [17]. Proposed mechanisms include DNA alteration, triggering oncogenic signaling pathways, producing tumor-promoting metabolites such as secondary bile acids, and inhibiting antitumor immunity [18]. Second, increased release of gastrin promoted by *H. pylori* infection could act as a mitogen, which might contribute to the development of CRC. This notion is suggested

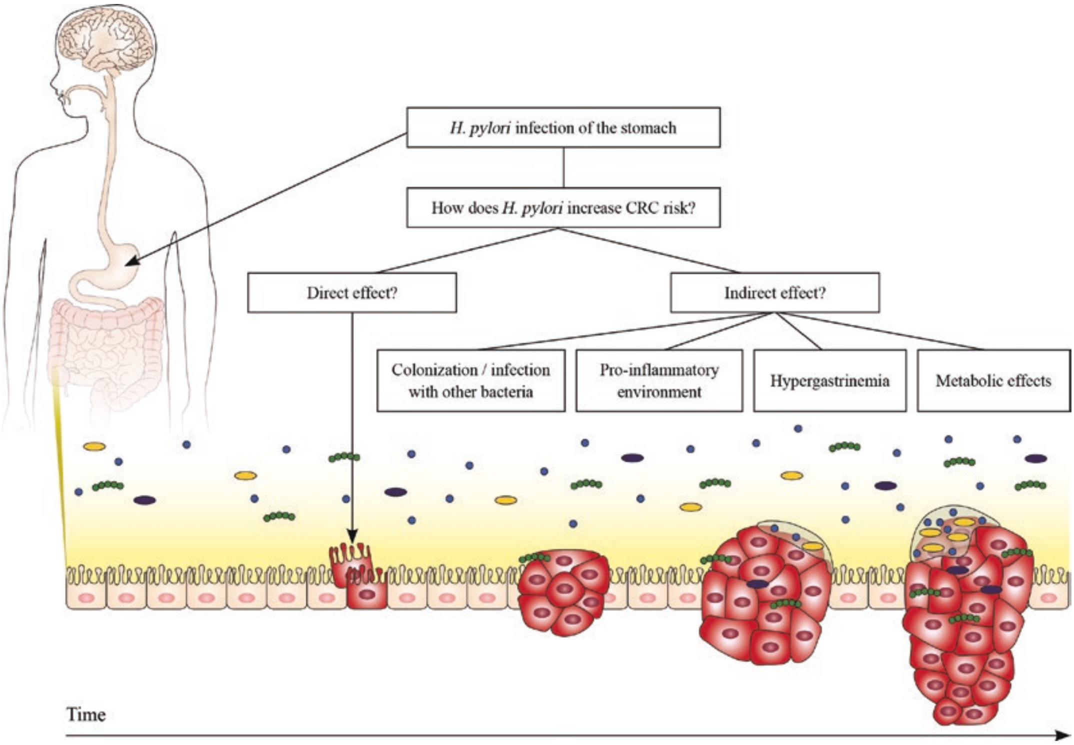
H. pylori infection of the stomach
How does *H. pylori* increase CRC risk?
Direct effect?
Indirect effect?
Colonization / infection with other bacteria
Pro-inflammatory environment
Hypergastrinemia
Metabolic effects
Time

Fig. 36.1 Potential mechanisms for direct and indirect causal effects of *H. pylori* on colorectal carcinogenesis. (Adapted from Butt and Epplein [10])

by the potential role of gastrin on epithelial cell growth and the prevention of apoptosis [19]. However, there have been conflicting results regarding the effects of gastrin in promoting the growth of colonic epithelium and CRC [19, 20]. Furthermore, other conditions with increased gastrin expression than *H. pylori* infection such as long-term proton pump inhibitor use, autoimmune gastritis, and gastrinoma are not associated with increased risk of CRC [21–23]. Another potential link behind this association is systemic inflammatory responses or metabolic disorders driven by *H. pylori*-induced chronic inflammation in the stomach [24]. However, it still needs to be clarified whether *H. pylori* could induce a pro-inflammatory condition and subsequently raise the risk of cancer in the lower GI tract.

36.3 Inflammatory Bowel Disease

36.3.1 Clinical Studies

The etiology of IBD is complex and multifactorial, involving immune dysfunction, genetic susceptibility, environmental factors, and changes in the gut microbial flora [25]. To date, many epidemiological and experimental investigations have reported an inverse correlation between *H. pylori* infection and IBD, suggesting that *H. pylori* may play a protective role in the development of IBD.

There was a recent study investigating the epidemiologic correlation between *H. pylori* and IBD, which included 59 studies on IBD prevalence and 127 studies on *H. pylori* prevalence [26]. According to the report, *H. pylori* prevalence was negatively correlated to the prevalence of IBD, Crohn's disease (CD), and ulcerative colitis (UC). A continent with more industrialized nations had higher rates of IBD and lower rates of *H. pylori*, whereas a continent with more developing countries had lower rates of IBD and higher rates of *H. pylori* [26] (Fig. 36.2). A meta-analysis on this association showed that the OR of positive *H. pylori* infection in IBD patients compared to controls was 0.44 (95% CI, 0.34–0.59), repeatedly indicating an inverse correlation between the two. Regarding the severity of disease, when the severe and moderate cases and mild and remission cases were compared, serious disease condition of IBD, CD, and UC was not associated with lower *H. pylori* infection. A prospective multicenter study in Korea including 316 IBD patients and matched controls also demonstrated similar results [27]. The prevalence of *H. pylori* infection was 25.3% in IBD patients, which was significantly lower than 52.5% in the control group ($p < 0.001$). In particular, the *H. pylori* prevalence was significantly lower in CD than in UC, and by age, this association was more pronounced in subjects under the age of 60.

Evidence is still lacking whether *H. pylori* eradication treatment can induce or aggravate IBD. There was a recent meta-analysis involving three studies on this subject, which showed that IBD patients were 1.41 times (95% CI, 1.25–1.58) more likely to relapse after *H. pylori* eradication compared to those without eradication [26]. However, in a prospective cohort study which investigated the influence of *H. pylori* eradication on disease activity of CD in six patients, there was no significant change in the disease activity measures such as Crohn's disease activity index, fecal calprotectin, or C-reactive protein after successful eradication [28]. Due to the paucity of evidences, it is challenging to draw conclusions about the effects of *H. pylori* eradication therapy on the development, recurrence, or aggravation of IBD.

36.3.2 Possible Mechanisms for Causal Effects

The epidemiological evidences show an inverse correlation between *H. pylori* infection and IBD, which gives rise to a speculation that *H.*

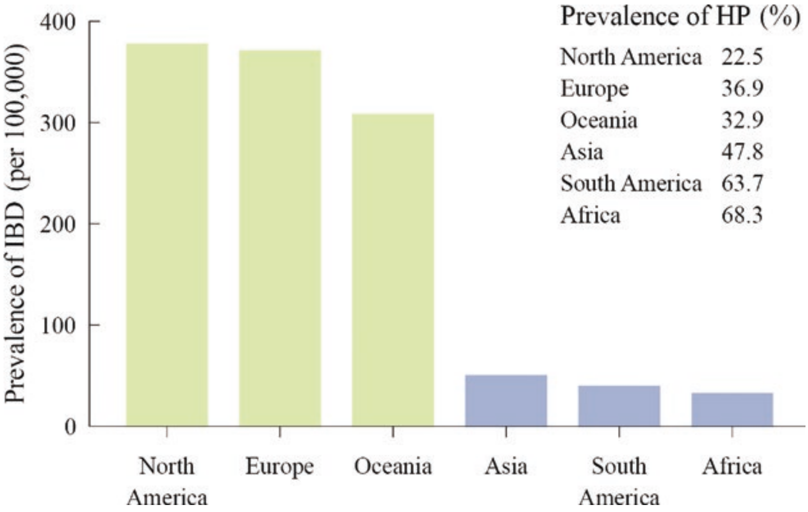

Fig. 36.2 Correlation between global *H. pylori* prevalence and IBD incidence by region. (Adapted from Zhong et al. [26])

pylori infection might play a protective role in the development of IBD. However, this association and its potential mechanisms have not been well-studied. A few suggested mechanisms are described below.

First, previous studies have reported that *H. pylori* infection can either induce autoimmunity by influencing the self-tolerance mechanism or show a protective impact against it. There are also some studies showing that *H. pylori* might have a protective effects against allergic diseases including asthma and atopy [29]. It is inferred that this protective effect of *H. pylori* against allergic diseases might be expressed by promoting Th1 immune response and suppressing Th2 immune response [30, 31]. *H. pylori* can even directly act on dendritic cells in the gastric mucosa to suppress the activity of T cells that cause allergic reactions [32]. Dendritic cells play a pivotal role in regulating the adaptive immunity and are shown to enter a functionally semi-mature state with a immune-tolerance phenotype by *H. pylori* [33]. In addition, chronic *H. pylori* infection induces differentiation of regulatory T cells (Tregs), which have important roles in the inhibition of autoimmunity and maintenance of immune homeostasis [34]. Systemic circulation of these Tregs might modulate the immune response, thereby affecting the development of IBD. Furthermore, TGF-β, IL-10, NLRP3, and IL-18 are thought to play an important role in the suppression of pro-inflammatory activities in IBD by *H. pylori* [35–37].

Second, *H. pylori* DNA may also play an important role in exerting a putative protective effect against IBD. An in vitro study showed that *H. pylori* DNA itself can also downregulate pro-inflammatory responses from dendritic cells and attenuate DSS-induced colitis [14]. In this study, administration of *H. pylori* DNA before the induction of DSS colitis significantly reduced the severity of colitis. Owyang et al. also demonstrated the ability of *H. pylori* DNA to downregulate inflammatory responses through interaction with mucosal dendritic cells in vitro and in vivo [38]. In this study, production of IL-12 and type I interferon was reduced by *H. pylori* DNA, and TLR-9 signaling pathway was important in this inhibitory effect. These results imply that the TLR-9 signaling pathway might be involved in the inhibitory effect of *H. pylori* in the pathogenesis of IBD. Furthermore, *CagA* gene, a renowned virulence gene of *H. pylori*, is associated with higher production of β-defensin. Since defensin is an antimicrobial peptide that plays a role in protecting the intestinal barrier and is closely associated with pathogenesis of IBD, this might also explain the potential protective effect of *H. pylori* against IBD [39]. There is a study showing a significant association between exposure to CagA seropositive *H. pylori* and decreased risk of IBD, which is not the case in CagA seronegative *H. pylori* infection [40].

Effect on intestinal microbiota is also another possible mechanism. According to literature, persistent *H. pylori* infection can significantly alter the composition of the gut microbiota, suggesting that *H. pylori* may regulate the microbial niche in the intestine and influence the development of IBD [41–44]. Data from both human and mouse research clearly suggest that the intestinal microbiota is crucial for the early development of the immune system in the gut, which may have an impact on IBD susceptibility [45]. Several studies have shown that certain symbiotic organisms can cause colitis in specific murine colitis models, which may be related to human IBD. In a mouse model of UC, for instance, *Proteus mirabilis* and *Klebsiella pneumoniae* were associated with colitis [46]. Another study showed that consumption of saturated fat influences the composition and diversity of the gut microbiota and promotes growth of a sulphite-reducing pathobiont, *Bilophila wadsworthia* [47]. This microbial change leads to pro-inflammatory Th-1 immune response and increased incidence of colitis in genetically susceptible mice. These findings show that microbial dysbiosis can perturb immune homeostasis and may be involved in the development of IBD in genetically susceptible hosts.

36.4 Irritable Bowel Syndrome

36.4.1 Clinical Studies

Changes in the gut microbiome have been proposed as a cause of IBS or chronic constipation. It is speculated that imbalance of microflora may cause visceral hypersensitivity or altered gastrointestinal motility through the gut-brain axis. There are various factors that can affect the gut microbiome, and as mentioned above, there are reports that *H. pylori* infection can be one of the factors.

In fact, there are not many studies on the association between *H. pylori* infection and IBS. Previous studies have shown conflicting results. While some of studies suggested that patients with IBS had higher incidence of *H. pylori* infection than healthy individuals, other studies found no significant differences between the subjects with or without IBS [48, 49]. In a recent meta-analysis which includes 10 studies reporting both the prevalence of *H. pylori* infection and IBS upto 2019, the OR for *H. pylori* in the IBS group was 1.10 (95% CI, 0.93–1.29), which was not significant [48]. This insignificance also persisted when subgroup analysis was performed only including the articles which defined IBS according to Rome criteria. However, the results of another recent meta-analysis are somewhat different [49]. It was characterized by inclusion of many non-English papers in China, comprising 27 studies in total. The rate of *H. pylori* infection was higher in patients with IBS than those without (OR 1.68; 95% CI, 1.29–2.18). Further subgroup analyses revealed that this significant association between IBS and *H. pylori* infection was present in Asian population, but not in non-Asian population.

In a recent big data cohort study using health insurance data for 14 years in Taiwan, a total of 2669 *H. pylori*-infected individuals and 10,676 non-*H. pylori*-infected individuals were followed to investigate the rate of IBS occurrence [50]. The cumulative incidence rate of IBD was significantly higher in the *H. pylori* infection without eradication group than in the non-*H. pylori*-infected group or *H. pylori* eradication group (Fig. 36.3). After adjusting for sex, age group, insurance premium, and preexisting comorbidi-

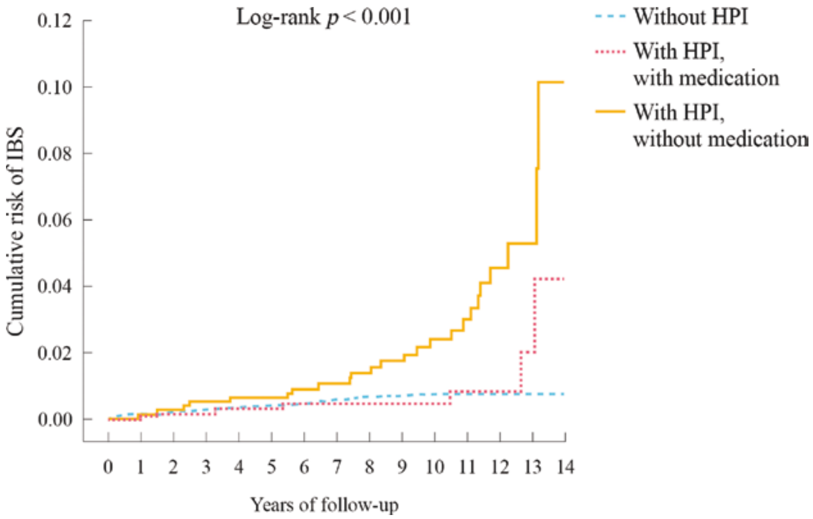

Fig. 36.3 Kaplan–Meier curve for the cumulative risk of IBS in individuals aged 14 or older stratified by *H. pylori* infection with the log-rank test (Adapted from Liang et al. [50]). Without HPI vs. With HPI, without medication: Log-rank $p < 0.001$. Without HPI vs. With HPI, with medication: Log-rank $p = 0.479$. With HPI, without medication vs. With HPI, with medication: Log-rank $p < 0.001$. *HPI Helicobacter pylori* infection, *IBS* irritable bowel syndrome

ties, *H. pylori* infection showed an increased risk of developing IBS compared to the control cohort (adjusted HR 3.108; 95% CI, 1.934–4.995). Interestingly, individuals with *H. pylori* infection who underwent aggressive eradication therapy showed a lower risk of developing IBS than those with *H. pylori* infection who did not receive eradication therapy (adjusted HR 0.464; 95% CI, 0.148–0.963).

There are some studies investigating the impact of *H. pylori* eradication on the improvement of IBS symptoms. In a meta-analysis, *H. pylori* eradication treatment significantly increased the rate of symptom improvement in IBS compared to the control group (RR 1.24; 95% CI, 1.10–1.39) [49].

36.4.2 Possible Mechanisms for Causal Effects

IBS is regarded as a disorder of gut-brain interaction according to the Rome IV criteria [51]. It could be implied from this concept that gut-brain interaction may be a bridge for establishing the relationship between *H. pylori* and IBS.

Local and systemic inflammatory response evoked by *H. pylori* infection might attribute to the development of IBS. Indeed, increased mast cell activation, elevated inflammatory markers, and stomach mucosal and neural remodeling have all been associated with *H. pylori* infection [24, 52, 53]. Increased mast cell activation and immune activity in the gut may be associated with visceral hypersensitivity, which is an important factor for symptom presentation in IBS [54, 55]. Through the release of cytotoxins (CagA, VacA) and the activation of the inflammatory process, *H. pylori* infection may directly cause changes in the function and morphology of the digestive tract [56]. Consequently, these changes could result in axon injury or stimulation, contributing to the development of IBS symptoms. Furthermore, *H. pylori* infection promotes release of various inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-8 [56]. Increased secretion of these cytokines can induce brain-gut axis dysfunction, which could worsen IBS symptoms.

Change in the gut microbiome induced by *H. pylori* is another proposed mechanism in the development of IBS. Alteration in the gut microbiota can change the production of neurotransmitters that impact the gut-brain axis [57]. Microbial dysbiosis in the gut can also cause dysmotility and hypersensitivity of the intestinal tract, by interfering with mucosal immunity and inducing the loss of epithelial layer [58].

36.5 Conclusions

There have been often controversial results regarding the association between *H. pylori* and disorders of lower GI tract, and most studies are focused on simple epidemiologic associations. Although it should be interpreted with caution, colorectal adenoma or cancer seems to have a positive correlation with *H. pylori* infection, while IBD might be inversely associated with *H. pylori* infection. There are relatively limited number of studies on the association between *H. pylori* and IBS, with conflicting results among studies. There could be differences in these potential associations depending on race/ethnics, especially for colorectal adenoma/cancer and IBS.

In an effort to better understand how a bacterium acting outside of its ecological niche-the gastric milieu-could either directly or indirectly affect the diseases in the lower GI tract, several plausible mechanisms have been proposed. These theories include virulence factors, metabolites, or the promotion of intestinal dysbiosis. However, there is paucity of evidence for a causal relationship between *H. pylori* and disorders of lower GI tract. Further research is also warranted on the potential mechanisms of this association.

References

1. Coelho LGV, Coelho MCF. *Helicobacter pylori* and colorectal neoplasms: a concise review. Arq Gastroenterol. 2021;58:114–9.
2. Nam JH, Hong CW, Kim BC, Shin A, Ryu KH, Park BJ, et al. *Helicobacter pylori* infection is an independent risk factor for colonic adenomatous neoplasms. Cancer Causes Control. 2017;28:107–15.

3. Lee JY, Park HW, Choi JY, Lee JS, Koo JE, Chung EJ, et al. *Helicobacter pylori* infection with atrophic gastritis is an independent risk factor for advanced colonic neoplasm. Gut Liver. 2016;10:902–9.
4. Butt J, Varga MG, Blot WJ, Teras L, Visvanathan K, Le Marchand L, et al. Serologic response to *Helicobacter pylori* proteins associated with risk of colorectal cancer among diverse populations in the United States. Gastroenterology. 2019;156:175–86.e2.
5. Wu Q, Yang ZP, Xu P, Gao LC, Fan DM. Association between *Helicobacter pylori* infection and the risk of colorectal neoplasia: a systematic review and meta-analysis. Color Dis. 2013;15:e352–64.
6. Zhao Y, Wang X, Wang Y. *Helicobacter pylori* infection and colorectal carcinoma risk: a meta-analysis. J Cancer Res Ther. 2016;12:15–8.
7. Yang F, Xu YL, Zhu RF. *Helicobacter pylori* infection and the risk of colorectal carcinoma: a systematic review and meta-analysis. Minerva Med. 2019;110:464–70.
8. Choi DS, Seo SI, Shin WG, Park CH. Risk for colorectal neoplasia in patients with *Helicobacter pylori* infection: a systematic review and meta-analysis. Clin Transl Gastroenterol. 2020;11:e00127.
9. Hu KC, Wu MS, Chu CH, Wang HY, Lin SC, Liu CC, et al. Decreased colorectal adenoma risk after *Helicobacter pylori* eradication: a retrospective cohort study. Clin Infect Dis. 2019;68:2105–13.
10. Butt J, Epplein M. *Helicobacter pylori* and colorectal cancer-a bacterium going abroad? PLoS Pathos. 2019;15:e1007861.
11. Grahn N, Hmani-Aifa M, Fransén K, Söderkvist P, Monstein HJ. Molecular identification of *helicobacter* DNA present in human colorectal adenocarcinomas by 16S rDNA PCR amplification and pyrosequencing analysis. J Med Microbiol. 2005;54:1031–5.
12. Soylu A, Ozkara S, Alis H, Dolay K, Kalayci M, Yasar N, et al. Immunohistochemical testing for *Helicobacter pylori* existence in neoplasms of the colon. BMC Gastroenterol. 2008;8:35.
13. Jones M, Helliwell P, Pritchard C, Tharakan J, Mathew J. *Helicobacter pylori* in colorectal neoplasms: is there an aetiological relationship? World J Surg Oncol. 2007;5:51.
14. Luther J, Owyang SY, Takeuchi T, Cole TS, Zhang M, Liu M, et al. *Helicobacter pylori* DNA decreases pro-inflammatory cytokine production by dendritic cells and attenuates dextran sodium sulphate-induced colitis. Gut. 2011;60:1479–86.
15. McClain MS, Beckett AC, Cover TL. *Helicobacter pylori* vacuolating toxin and gastric cancer. Toxins (Basel). 2017;9:316.
16. Shin CM. Alternations of gastric microbiota with mucosal atrophy and intestinal metaplasia. Korean J Helicobacter Up Gastrointest Res. 2020;20:187–95.
17. Kim J, Lee HK. Potential role of the gut microbiome in colorectal cancer progression. Front Immunol. 2021;12:807648.
18. Pandey H, Tang DWT, Wong SH, Lal D. Gut microbiota in colorectal cancer: biological role and therapeutic opportunities. Cancers (Basel). 2023;15:866.
19. Thorburn CM, Friedman GD, Dickinson CJ, Vogelman JH, Orentreich N, Parsonnet J. Gastrin and colorectal cancer: a prospective study. Gastroenterology. 1998;115:275–80.
20. Robertson DJ, Sandler RS, Ahnen DJ, Greenberg ER, Mott LA, Cole BF, et al. Gastrin, *Helicobacter pylori*, and colorectal adenomas. Clin Gastroenterol Hepatol. 2009;7:163–7.
21. Lee JK, Merchant SA, Schneider JL, Jensen CD, Fireman BH, Quesenberry CP, et al. Proton pump inhibitor use and risk of gastric, colorectal, liver, and pancreatic cancers in a community-based population. Am J Gastroenterol. 2020;115:706–15.
22. Lahner E, Capasso M, Carabotti M, Annibale B. Incidence of cancer (other than gastric cancer) in pernicious anaemia: a systematic review with meta-analysis. Dig Liver Dis. 2018;50:780–6.
23. Orbuch M, Venzon DJ, Lubensky IA, Weber HC, Gibril F, Jensen RT. Prolonged hypergastrinemia does not increase the frequency of colonic neoplasia in patients with Zollinger-Ellison syndrome. Dig Dis Sci. 1996;41:604–13.
24. Jackson L, Britton J, Lewis SA, McKeever TM, Atherton J, Fullerton D, et al. A population-based epidemiologic study of *Helicobacter pylori* infection and its association with systemic inflammation. Helicobacter. 2009;14:108–13.
25. Zhang YZ, Li YY. Inflammatory bowel disease: pathogenesis. World J Gastroenterol. 2014;20:91–9.
26. Zhong Y, Zhang Z, Lin Y, Wu L. The relationship between *Helicobacter pylori* and inflammatory bowel disease. Arch Iran Med. 2021;24:317–25.
27. Song MJ, Park DI, Hwang SJ, Kim ER, Kim YH, Jang BI, et al. The prevalence of *Helicobacter pylori* infection in Korean patients with inflammatory bowel disease, a multicenter study. Korean J Gastroenterol. 2009;53:341–7.
28. Lahat A, Kopylov U, Neuman S, Levhar N, Yablecovitch D, Avidan B, et al. *Helicobacter pylori* prevalence and clinical significance in patients with quiescent Crohn's disease. BMC Gastroenterol. 2017;17:27.
29. Park JY. *Helicobacter pylori* and immune-mediated disorders. Korean J Helicobacter Up Gastrointest Res. 2020;20:29–37.
30. Mai J, Liang B, Xiong Z, Ai X, Gao F, Long Y, et al. Oral administration of recombinant Bacillus subtilis spores expressing *Helicobacter pylori* neutrophil-activating protein suppresses peanut allergy via up-regulation of Tregs. Clin Exp Allergy. 2019;49:1605–14.
31. Kyburz A, Fallegger A, Zhang X, Altobelli A, Artola-Boran M, Borbet T, et al. Transmaternal *Helicobacter pylori* exposure reduces allergic airway inflammation in offspring through regulatory T cells. J Allergy Clin Immunol. 2019;143:1496–512.e11.
32. Oertli M, Sundquist M, Hitzler I, Engler DB, Arnold IC, Reuter S, et al. DC-derived IL-18 drives Treg differentiation, murine *Helicobacter pylori*-specific immune tolerance, and asthma protection. J Clin Invest. 2012;122:1082–96.

33. Kao JY, Zhang M, Miller MJ, Mills JC, Wang B, Liu M, et al. *Helicobacter pylori* immune escape is mediated by dendritic cell-induced Treg skewing and Th17 suppression in mice. Gastroenterology. 2010;138:1046–54.
34. Li C, Jiang P, Wei S, Xu X, Wang J. Regulatory T cells in tumor microenvironment: new mechanisms, potential therapeutic strategies and future prospects. Mol Cancer. 2020;19:116.
35. Powrie F, Carlino J, Leach MW, Mauze S, Coffman RL. A critical role for transforming growth factor-beta but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB(low) CD4+ T cells. J Exp Med. 1996;183:2669–74.
36. Kim DJ, Park JH, Franchi L, Backert S, Núñez G. The cag pathogenicity Island and interaction between TLR2/NOD2 and NLRP3 regulate IL-1β production in *Helicobacter pylori* infected dendritic cells. Eur J Immunol. 2013;43:2650–8.
37. Hitzler I, Sayi A, Kohler E, Engler DB, Koch KN, Hardt WD, et al. Caspase-1 has both proinflammatory and regulatory properties in *helicobacter* infections, which are differentially mediated by its substrates IL-1β and IL-18. J Immunol. 2012;188:3594–602.
38. Owyang SY, Luther J, Owyang CC, Zhang M, Kao JY. *Helicobacter pylori* DNA's anti-inflammatory effect on experimental colitis. Gut Microbes. 2012;3:168–71.
39. Ramasundara M, Leach ST, Lemberg DA, Day AS. Defensins and inflammation: the role of defensins in inflammatory bowel disease. J Gastroenterol Hepatol. 2009;24:202–8.
40. Tepler A, Narula N, Peek RM Jr, Patel A, Edelson C, Colombel JF, et al. Systematic review with meta-analysis: association between *Helicobacter pylori* CagA seropositivity and odds of inflammatory bowel disease. Aliment Pharmacol Ther. 2019;50:121–31.
41. Iino C, Shimoyama T, Chinda D, Sakuraba H, Fukuda S, Nakaji S. Influence of *Helicobacter pylori* infection and atrophic gastritis on the gut microbiota in a Japanese population. Digestion. 2020;101:422–32.
42. Bühling A, Radun D, Müller WA, Malfertheiner P. Influence of anti-*helicobacter* triple-therapy with metronidazole, omeprazole and clarithromycin on intestinal microflora. Aliment Pharmacol Ther. 2001;15:1445–52.
43. Li L, Zhou X, Xiao S, Ye F, Zhang G. The effect of *Helicobacter pylori* eradication on the gastrointestinal microbiota in patients with duodenal ulcer. J Gastrointestin Liver Dis. 2016;25:139–46.
44. Chen CC, Liou JM, Lee YC, Hong TC, El-Omar EM, Wu MS. The interplay between *Helicobacter pylori* and gastrointestinal microbiota. Gut Microbes. 2021;13:1–22.
45. Yang Z, Liu X, Wu Y, Peng J, Wei H. Effect of the microbiome on intestinal innate immune development in early life and the potential strategy of early intervention. Front Immunol. 2022;13:936300.
46. Garrett WS, Gallini CA, Yatsunenko T, Michaud M, DuBois A, Delaney ML, et al. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. Cell Host Microbe. 2010;8:292–300.
47. Devkota S, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A, et al. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10−/− mice. Nature. 2012;487:104–8.
48. Kim YA, Cho YJ, Kwak SG. The association between *Helicobacter pylori* infection and irritable bowel syndrome: a meta-analysis. Int J Environ Res Public Health. 2020;17:2524.
49. Wang C, Yin Y, Wang L, Guo X, Liu L, Qi X. Association between *Helicobacter pylori* infection and irritable bowel syndrome: a systematic review and meta-analysis. Postgrad Med J. 2021;99:166.
50. Liang CM, Hsu CH, Chung CH, Chen CY, Wang LY, Hsu SD, et al. Risk for irritable bowel syndrome in patients with *Helicobacter pylori* infection: a nationwide population-based study cohort study in Taiwan. Int J Environ Res Public Health. 2020;17:3737.
51. Drossman DA, Hasler WL. Rome IV-functional GI disorders: disorders of gut-brain interaction. Gastroenterology. 2016;150:1257–61.
52. Montemurro P, Nishioka H, Dundon WG, de Bernard M, Del Giudice G, Rappuoli R, et al. The neutrophil-activating protein (HP-NAP) of *Helicobacter pylori* is a potent stimulant of mast cells. Eur J Immunol. 2002;32:671–6.
53. Stead RH, Hewlett BR, Lhotak S, Colley ECC, Dixon MF. Do gastric mucosal nerves remodel in *H. pylori* gastritis? In: Richard HH, Tytgat GNJ, editors. Helicobacter pylori: basic mechanisms to clinical cure. Berlin/Heidelberg, Germany: Springer; 1994. p. 281–91.
54. Gerards C, Leodolter A, Glasbrenner B, Malfertheiner P. *H. pylori* infection and visceral hypersensitivity in patients with irritable bowel syndrome. Dig Dis. 2001;19:170–3.
55. Ohman L, Simrén M. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. Nat Rev Gastroenterol Hepatol. 2010;7: 163–73.
56. Budzyński J, Kłopocka M. Brain-gut axis in the pathogenesis of *Helicobacter pylori* infection. World J Gastroenterol. 2014;20:5212–25.
57. Peuhkuri K, Vapaatalo H, Korpela R. Even low-grade inflammation impacts on small intestinal function. World J Gastroenterol. 2010;16:1057–62.
58. Stanghellini V, Barbara G, de Giorgio R, Tosetti C, Cogliandro R, Cogliandro L, et al. *Helicobacter pylori*, mucosal inflammation and symptom perception—new insights into an old hypothesis. Aliment Pharmacol Ther. 2001;15 (Suppl 1):28–32.

37

Extraintestinal Manifestations of *H. pylori* Infection: Neurologic Disease

Soo In Choi

Abstract

An important role of the gut microbiota in neurologic diseases is emerging. The microbiota-derived metabolites and their products can induce neuroinflammation through the microbiota-gut-brain axis (MGBA) via the enteric nerve, vagus nerve, endocrine, or immune pathways. *Helicobacter pylori* (*H. pylori*) can also be involved in the nervous system via MGBA by inducing pro-inflammatory process and gut dysbiosis during gastric colonization. In addition, gastric atrophy as a result of chronic infection can cause microelement deficiency such as vitamin B12, which affects brain function. Previous published epidemiologic studies and meta-anaysis have reported that *H. pylori* infection may be related to the development and prognosis of various neurologic diseases such as Parkinson's disease, Alzheimer's disease, Guillain-Barré syndrome, multiple sclerosis, and ischemic stroke. However, data are still lacking and the association between *H. pylori* infection and neurologic diseases remains unclear. To understand the effect and mechanism of *H. pylori* infection on neurologic diseases, more research will be needed.

Keywords

Helicobacter pylori · Neurologic disease
Nervous system diseases

37.1 Introduction

It became clear that the gut microbiome can communicate with the central nervous system (CNS) via several pathways, together called the microbiota-gut-brain axis (MGBA) [1]. Many animal and epidemiological studies have implicated that the gut microbiota can affect various neurodegenerative and neurodevelopmental diseases via MGBA [2]. The hypothesis for the association between *H. pylori* infection and neurologic diseases is based on that *H. pylori* infection may affect the CNS via MBGA by inducing pro-inflammatory responses, gut dysbiosis, and microelement deficiency such as vitamin B12. This chapter is going to introduce the pathophysiology of *H. pylori* infection on neurologic diseases and review the previous epidemiological researches on the relationship between *H. pylori* and neurologic diseases.

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37.2 Pathophysiology

37.2.1 The Microbiota-Gut-Brain Axis

The gut-brain axis is a complex network in which the CNS and enteric nervous system (ENS) are connected bidirectionally. It is known that the gut microbiota can modulate the CNS functions through the ENS, vagus nerve, circulatory system, or immune system to reach the brain, involving various neuroactive compounds including the microbial-derived metabolites, microbial-derived products, peptides, gut hormones, and neuroactive substances [3]. These pathways are collectively referred to as MGBA. Many studies have implicated the gut microbiota in neurodevelopment and neurodegeneration of numerous conditions such as multiple sclerosis, Parkinson’s Disease (PD), Alzheimer’s Disease (AD), ischemic stroke, depression, anxiety, stress, and schizophrenia, via the MGBA [4, 5]. The effect of *H. pylori* infection on the CNS is also based on its role within the MGBA [6]. The brief mechanisms of gut microbiota involved in the MGBA are as follows. (1) microbial-derived metabolites (e.g., short-chain fatty acids) can contribute to the MGBA by exerting their effects through receptor-mediated interactions on various host tissues or cells [3]. (2) microbial-derived products (e.g., lipopolysaccharide) play an important role in the MGBA communication, often through interactions with the toll-like receptors in the ENS and CNS that can sense the products through recognized molecular patterns [3]. (3) gut microbiota involves in the production and release of gut hormones (e.g., serotonin), which can promote peripheral immune system and send signals to the CNS through activation of vagal sensory afferents [3]. (4) gut microbiota can regulate the synthesis and release of neuroactive molecules or their precursors including dopamine, noradrenaline, acetylcholine, histamine, melatonin, and gamma-aminobutyric acid [3]. (5) gut microbiota and their components can infiltrate the gut associated lymphoid tissues and the bloodstream, interact with various immune cells, and upregulate inflammatory cytokine levels, causing the blood-brain barrier (BBB) dysfunction [5], and directly influence the CNS after being transported across the BBB. After all, the interactions between the gut microbiota and the CNS may elicit a variety of physiological, immune, and CNS responses, and it is thought to be more complex in relation to *H. pylori* infection.

37.2.2 Pathophysiology of *H. pylori* Infection on the MGBA

The pathophysiological mechanisms for the effect of *H. pylori* infection on MGBA are as follows; (1) direct neurotoxicity, (2) pro-inflammatory process, (3) change in neurotransmitters in the gastric mucosa and the CNS, (4) reduced absorption of vitamin B12 due to atrophic change of gastric mucosa, and (5) dysbiosis of gastric microbiota induced by *H. pylori* infection. Figure 37.1 summarized these mechanisms.

37.2.2.1 Route of *H. pylori* Entering the CNS

Three possible routes for *H. pylori* to enter the brain have been proposed. First is the oro-naso-olfactory pathway, which allows bacteria to enter the brain through the epithelium of the mouth or nasopharynx [7]. Second is a pathway of invasion through the disrupted BBB by pro-inflammatory cytokines, while *H. pylori* avoiding phagocytosis and surviving in the bloodstream, which is known as the “Trojan horse theory” [7]. Third is the retrograde axonal transport pathways, through which pathogens can affect the brain by conveying materials from axon to cell body [5, 7]. However, the exact mechanisms or entry points of invasion remain unclear.

37.2.2.2 Neuroinflammation by *H. pylori* Infection

As is known, *H. pylori* can survive in the stomach with colonization for a long period of time, manipulating innate immune receptors and inhibiting effector T-cell responses through certain bacterial virulence factors [1]. Chronic *H. pylori* infection on the gastric mucosa can cause neurotoxic effects directly through the cytotoxic

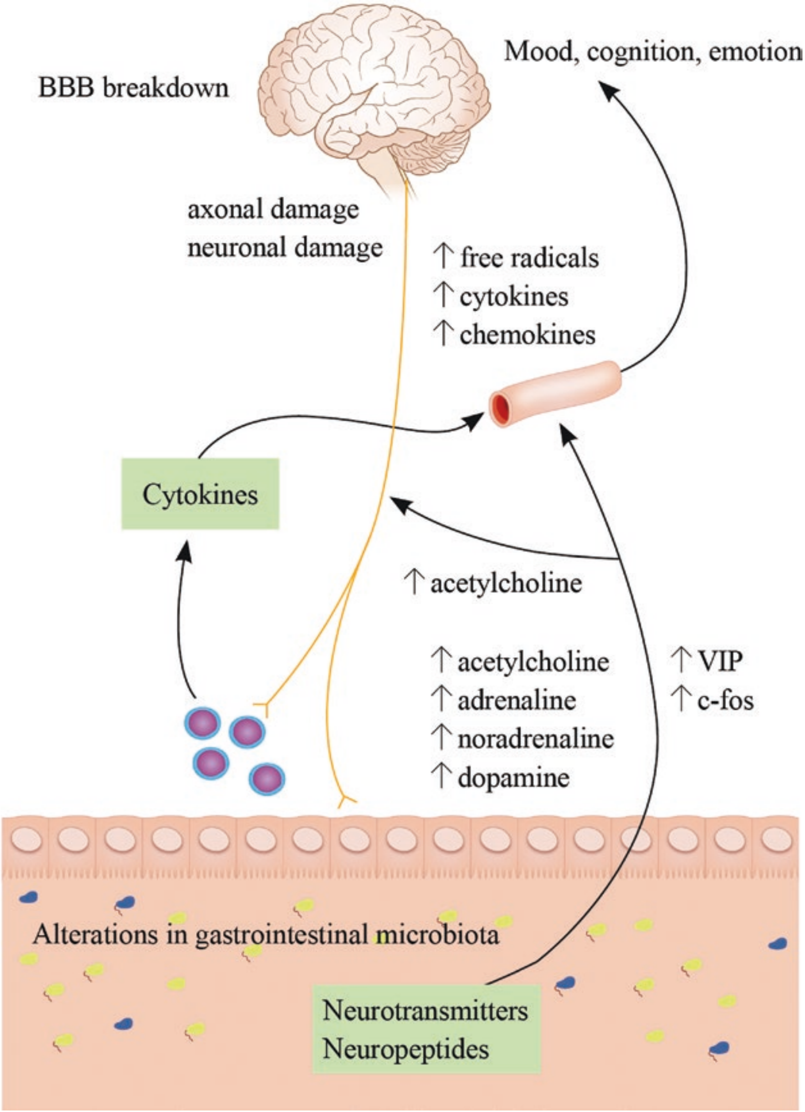
BBB breakdown
Mood, cognition, emotion
axonal damage
neuronal damage
↑ free radicals
↑ cytokines
↑ chemokines
Cytokines
↑ acetylcholine
↑ acetylcholine
↑ adrenaline
↑ noradrenaline
↑ dopamine
↑ VIP
↑ c-fos
Alterations in gastrointestinal microbiota
Neurotransmitters
Neuropeptides

Fig. 37.1 Pathophysiologic mechanism of *H. pylori* infection on the microbiota-gut-brain axis. (Adapted from Gorlé et al. [1])

releases such as CagA and VacA and activate the inflammatory process. The most important known virulence factor that evokes an immune response by the host is VacA [7]. VacA stimulates bone marrow-derived mast cells to induce local secretion of multiple degranulated chemotactic factors and pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, IL-10, IL-12, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, which might reach the circulation causing a systemic effect [7]. For example, IL-8 and IFN-γ can mediate the responses to bacterial infection and to autoimmune disease [8]. TNF-α disrupts the integrity of the BBB by activating matrix metalloproteinases [7]. In addition, the long time exposure to *H. pylori*-neutrophil-activating protein (HP-NAP), a pro-inflammatory protein commonly found in *H. pylori*-related gastritis, can induce migration and activation of neutrophils and increase the production of free radicals [7]. Eventually, the persistence of noticeable local and systemic concentrations of these pro-inflammatory factors can induce the BBB disruption and penetration of immunotoxic substances to the CNS, resulting in neuroinflammation and direct neurotoxic effects including demyelination, inflammation, and edema processes [1, 7]. These inflammatory processes may continue until the bacteria are eradicated.

37.2.2.3 Dysbiosis by *H. pylori* Infection

Recent studies have shown a noticeable correlation between the imbalance of the gut microbiome and neurologic diseases [5]. The microbial imbalance leads to changes in secretion of their metabolites and products, such as short-chain fatty acids, tryptophan, and bile acid metabolites, known to be involved in various aspects of brain function and behavior [4]. Many studies have demonstrated differences in the abundance of microbiota in various neurologic diseases compared to controls [4]. For example, changes in the abundance of some gut bacteria such as *Prevotellacea* and *Enterobacteriaceae* have been identified in PD [4]. As described in other chapters, *H. pylori* colonization in the stomach leads to an imbalance of the gut microbiome [7], and consequently, it can be potential risk for the development and exacerbation of various neurological diseases.

37.2.2.4 Effects of *H. pylori* on Neurotransmitters

Neurotransmitters are actively involved in various brain functions including movement, emotion, learning, and memory [9]. The released inflammatory mediators as a result of *H. pylori* infection can affect the functions of the hypothalamus and brainstem by disrupting the neuroendocrine–immune system and activating the hypothalamic–pituitary–adrenal axis, which is associated with increased secretion of neurotransmitters, such as cortisol and adrenaline [7]. It has also been proven that *H. pylori* infection can lead to the release of several other neurotransmitters such as acetylcholine, noradrenaline, dopamine, adrenaline, and serotonin [7]. The disturbances of homeostasis in these neurotransmitters, consequently, can lead to alterations in brain function and influence neurologic and psychological disorders such as AD, PD, autism, and schizophrenia [9].

37.2.2.5 Microelement Deficiency

Chronic *H. pylori* infection induces gastric mucosal atrophy and leads to reduced absorption of vitamin B12. Vitamin B12, a cofactor in myelin formation, can significantly influence the function of the nervous system by producing neurotrophic and immunomodulating effect in the nervous system [7]. It is known that vitamin B12 hypovitaminosis can cause pathological changes in the white and gray matter of the brain and is associated with neurological manifestations, such as sensorimotor polyneuropathy, subacute complex degeneration of the spinal cord, cognitive impairment, and optic neuropathy [7]. In addition, decreased levels of vitamin B12 have been reported in patients with multiple sclerosis, and avitaminosis of vitamin B12 has been suggested to indirectly increase the risk of ischemic stroke and cognitive disorders such as AD, by increasing homocysteine levels regarding vitamin B12 metabolism, which generate free radicals and oxidative stress causing vascular damage and lipid peroxidation [7, 8].

37.3 Related Neurologic Diseases

37.3.1 Parkinson's Disease

PD is an idiopathic progressive degenerative disease in which neuronal cytoplasmic inclusions such as alpha-synuclein accumulate and cause the loss of dopaminergic neurons. Since Altschuler et al. first suggested a relationship between *H. pylori* and PD [10], a number of studies have reported a positive correlation between *H. pylori* infection and PD (Table 37.1). In a meta-analysis including eight studies covering 33,125 PD patients published in 2017, the pooled odds ratio (OR) of PD in *H. pylori*-infected person was 1.59 (95% confidence interval [CI], 1.37–1.85) and in subgroup analyses, the combined OR was higher in Asia compared to that in Europe (OR 1.96; 95% CI, 1.23–3.12 vs. OR 1.55; 95% CI, 1.32–1.82) [11]. In a meta-analysis including ten studies covering 5043 PD patients and 23,449 healthy controls published in 2018,

Table 37.1 Association between *H. pylori* and neurologic disease

Disease	Year	Country	Study design	Sample size	Results	References
Parkinson's disease (PD)	1999	United Kingdom	Case-control	Case = 33 Control = 78	Positive association	[56]
	2000	United Kingdom	Case-control	Case = 105 Control = 210	Positive association	[57]
	2012	Denmark	Case-control	Case = 4484 Control = 22,416	Positive association	[58]
	2018	China	Cohort	N = 18,210	Positive association	[13]
	2017	China	Meta-analysis	8 studies, 33,125 participants	Positive association	[11]
	2018	Greece	Meta-analysis	10 studies, 28,492 participants	Positive association	[12]
Alzheimer's disease (AD)	2003	Sweden	Case-control	Case = 47 Control = 101	No association	[59]
	2004	Italy	Case-control	Case = 30 Control = 30	Positive association	[17]
	2006	Greece	Case-control	Case = 50 Control = 30	Positive association	[18]
	2011	Japan	Case-control	Case = 385 Control = 97	No association	[19]
	2014	China	Cohort	N = 83,965	No association	[20]
	2015	Greece	Case-control	Case = 36 Control = 31	Positive association	[60]
	2018	Netherlands	Cohort	N = 4215	No association	[21]
	2018	United States	Cohort	N = 5927	Positive association	[22]
	2021	China	Meta-analysis	10 studies, 96,561 participants	No association	[23]

(continued)

Table 37.1 (continued)

Disease	Year	Country	Study design	Sample size	Results	References
Demyelinating diseases; multiple sclerosis (MS) and neuromyelitis optica spectrum disorders (NMOSD)	2007	Japan	Case-control	Case = 105 Control = 85	Negative association	[28]
	2009	Japan	Case-control	Case = 162 Control = 85	Positive association	[29]
	2012	Japan	Case-control	Case = 261 Control = 367	Positive association	[61]
	2013	China	Case-control	Case = 118 Control = 27	Positive association	[30]
	2013	Iran	Case-control	Case = 163 Control = 150	Negative association	[62]
	2015	United Kingdom	Case-control	Case = 71 Control = 42	Negative association	[63]
	2015	Greece	Case-control	Case = 44 Control = 20	Positive association	[31]
	2015	India	Case-control	Case = 139 Control = 278	Negative association	[64]
	2015	Australia	Case-control	Case = 550 Control = 299	Negative association	[65]
	2017	Greece	Case-control	Case = 139 Control = 68	Positive association	[36]
	2020	Iran	Case-control	Case = 97 Control = 95	Positive association	[66]
	2020	Iran	Case-control	Case = 39 Control = 39	Positive association	[67]
	2022	Iran	Meta-analysis	22 studies, 4806 participants	Positive association	[32]

Disease	Year	Country	Study design	Sample size	Results	References
Guillain–Barré syndrome (GBS)	2003	Italy	Case-control	Case = 33 Control = 30	No association	[68]
	2004	Japan	Case-control	Case = 12 Control = 42	Possible positive association	[69]
	2005	Greece	Case-control	Case = 13 Control = 20	Possible positive association	[38]
	2010	Iran	Case-control	Case = 15 Control = 15	Positive association	[39]
	2020	Greece	Meta-analysis	6 studies, 430 participants	Positive association	[40]
Ischemic stroke	1998	United Kingdom	Case-control	Case = 238 Control = 119	Positive association	[70]
	2001	Germany	Case-control	Case = 109 Control = 82	Positive association	[71]
	2001	Germany	Case-control	Case = 145 Control = 260	Positive association	[72]
	2002	Italy	Case-control	Case = 80 Control = 320	Positive association	[73]
	2002	Italy	Case-control	Case = 138 Control = 151	Positive association	[74]
	2003	United Kingdom	Case-control	Case = 467 Control = 388	Positive association	[75]
	2004	Italy	Case-control	Case = 105 Control = 130	Positive association	[76]
	2004	Germany	Case-control	Case = 119 Control = 229	Positive association	[77]
	2005	Japan	Case-control	Case = 62 Control = 143	Positive association	[48]
	2008	Iran	Case-control	Case = 81 Control = 43	No association	[78]
	2008	Italy	Case-control	Case = 106 Control = 106	Positive association	[45]
	2010	United States	Cohort	*N* = 1625	Positive association	[79]
	2012	Germany	Cohort	*N* = 9953	No association	[80]
	2012	China	Meta-analysis	13 studies, 4041 participants	Positive association	[46]
	2014	China	Cohort	N = 69,660	Positive association	[81]
	2021	Egypt, Saudi Arabia, United States	Meta-analysis	30 studies, 73,135 participants	Positive association	[47]

H. pylori infection prevalence was significantly higher in PD patients than in healthy controls (OR 1.47; 95% CI, 1.27–1.70; Pz < 0.00001) [12]. In addition, this report showed that *H. pylori* infection was related to the higher disease severity in the unified Parkinson's disease rating scale (UPDRS) score (standardized mean differences [SMD] 0.33; 95% CI, 0.12–0.54; Pz = 0.003) [12]. In a large cohort study conducted in Taiwan in 2018, 9105 *H. pylori* infection cases and 9105 controls were compared for 12 years through a propensity scoring match [13]. This study reported that the *H. pylori*-infected group had a significantly higher incidence of PD with a hazard ratio (HR) of 2.29 (95% CI, 1.44–3.66), and it was only significant in those aged 60 years or older (HR 2.53; 95% CI, 1.47–4.35) [13]. The result of so far studies indicates that *H. pylori* infection may be strongly associated with an increased risk of PD.

A possible mechanism by which *H. pylori* infection is involved in PD suggested that *H. pylori* infection permits immunologically activated molecules to pass the BBB, causing damage to dopaminergic cells in the brain [14]. Continuous secretion of pro-inflammatory cytokines induced by chronic *H. pylori* infection disrupts the BBB and increases the expression of toll-like receptors in microglial cells responsible for neuroinflammation in the CNS, resulting in destroying the dopamine releasing neurons [15]. In addition, Schulz et al. proposed a hypothesis based on their experiments that cholesterol glucosides generated by *H. pylori* infection may act as neurotoxins, leading to the degeneration of dopaminergic neurons [16].

37.3.2 Alzheimer's Disease

AD is a neurodegenerative disease characterized by the loss of neurons due to deposition of beta amyloid in the cerebral cortex and subcortical regions of the brain. The inflammatory response is known to play an important role in the pathophysiology of AD [8]. Many studies have been proposed that *H. pylori* infection may be involved in the pathogenesis and development of AD; however, the association between *H. pylori* infection and AD is still controversial (Table 37.1). In small group studies conducted in 2004–2010, the patients with AD reported a higher *H. pylori* infection rate than control groups [17, 18]. Subsequent studies with larger numbers of patients reported conflicting results regarding the relationship between AD and *H. pylori* infection. A study published in 2011 comparing 385 AD patients and 97 controls in Japan reported that there was no difference in *H. pylori* infection between the two groups [19]. In a study covering 4275 dementia patients, Huang et al. reported that *H. pylori* infection was associated with non-AD, but not with AD [20]. A prospective cohort study conducted in Netherlands followed 4215 patients for a median of 13.3 years; they reported that there was no significant correlation between *H pylori* infection and AD [21]. However, a large-scale cohort study using the US National Registris published in 2018 reported that there was a significant difference in the incidence of AD according to *H. pylori* seropositivity (7.9% vs. 11. 3%; $p = 0.003$), and *H. pylori* infection and AD incidence showed significant correlations, especially in men (HR 1.56; 95% CI, 1.12–2.18; $p = 0.008$) and groups with high socioeconomic status (HR 1.43; 95% CI, 1.03–1.99) [22]. They reported that *H. pylori* infection was also associated with mortality from AD (HR 1.71; 95% CI, 0.94–3.11; $p = 0.08$) [22]. A meta-analysis including ten studies published in 2020 reported a significant association between *H. pylori* infection and all-cause dementia (pooled relative risk [RR] 1.36; 95% CI, 1.11–1.67), but there was no association between *H. pylori* infection and the risk of developing AD [23]. However, most of the studies so far are observational studies, and there are limitations in that there is heterogeneity between studies and that the confounding factors cannot be controlled. Therefore, additional randomized controlled trials will be needed to prove their causal relationship.

The possible mechanisms by which *H. pylori* infection can affect the neurodegenerative process in AD have been proposed as follows: (1) *H. pylori* infection-induced inflammatory responses influencing the apoptotic process which is an important form of cell death in AD [24], and (2) increased homocysteine levels, which cause endothelial damage and neurodegeneration due to reactive oxygen species (ROS) and peroxide lipid production, in relation to progressed atrophic gastritis and vitamin B12 malabsorption [25, 26].

37.3.3 Demyelinating Diseases of the CNS; Multiple Sclerosis and Neuromyelitis Optica Spectrum Disorders

Multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD) are autoimmune demyelinating disorders of the CNS that repeatedly affect the brain, optic nerve, or spinal cord. In neuromyelitis optica (NMO), water channel aquaporin-4 (AQP-4) has been demonstrated as the main antigen [27], but a specific antigen for MS is not yet known. Although the exact pathogenesis of MS remains unclear, there is a hypothesis that environmental factors may be involved and that bacterial infections can prevent MS outcomes or, conversely, be involved in the pathogenesis of the disease [7]. Epidemiologic clinical studies on the correlation between *H. pylori* infection and these demyelinating diseases have been reported (Table 37.1), but the relationships are still unclear. In a study of Japan published in 2007 covering the 53 patients with conventional MS (CMS), 52 patients with opticospinal MS (OSMS), and 85 control subjects, the CMS patients had a significantly lower *H. pylori* seropositivity than OSMS patients or controls, and the *H. pylori* seropositivity showed the inverse correlations with the fulfillment of McDonald criteria and the Expanded Disability Status Scale (EDSS) score, which are used in diagnosis and quantifying disability in MS [28]. Based on these results, they suggested that *H. pylori* infection could be a protective factor for MS. However, in a study including 162 patients with MS and NMOSD analyzed according to anti-AQP-4 antibody status, anti-AQP-4 antibody-positive MS/NMO patients showed significantly higher *H. pylori* seropositivity rates than anti-AQP-4 antibody-negative CMS patients (70.4% vs. 26.5%) [29]. In addition, among *H. pylori*-infected groups, anti-HP-NAP antibody was significantly more common in anti-AQP-4 antibody-positive MS patients than healthy subjects (36.8% vs. 2.8%), and anti-HP-NAP antibody levels showed a significant positive correlation with the EDSS scores ($R = 0.405$; $p = 0.0299$) [29]. Based on this, they suggested that in MS/NMO patients, HP-NAP may be associated with anti-AQP-4 antibody-related neural damage. Another study involving 52 NMO patients and 42 MS patients reported that *H. pylori* seropositivity was significantly higher in NMO spectrum patients than in MS patients or controls ($p < 0.05$), and particularly, in AQP-4 antibody-positive patients than in AQP-4 antibody-negative patients (93.1% vs. 80%; $p = 0.038$) [30]. In a study published in Greece in 2015 comparing 44 MS patients with 20 anemic controls, *H. pylori* infection rates were more frequent in MS patients than 20 matched anemic controls (86.4% vs. 50%) [31]. A recent meta-analysis including 22 studies published in 2022 reported that the OR of *H. pylori* infection to the development of MS was 0.82 (95% CI, 0.58–1.17), implying that *H. pylori* infection might be a risk factor rather than a protective factor in the development of MS [32].

The protective effect of *H. pylori* infection on the development of MS has suggested that certain microbial infections including *H. pylori* in infancy may have protective effects against autoimmune diseases in regard to the hygiene hypothesis [33, 34]. In contrast, as a mechanism by which *H. pylori* infection contributes to the pathogenesis of MS, nerve damage induced by the cross-reaction resulting in immune response to *H. pylori* infection has been hypothesized. As related factors, HP-NAP, *H. pylori* heat shock proteins (Hsp), and VacA-related antigen have been proposed [29, 35, 36], and several *H. pylori* antigens have been considered to be important for loss of immunological tolerance to myelin antigens. The epitopes of Hsp have been suggested to induce an immune response through cross-reactivity, thereby losing immunological tolerance to myelin antigens and causing autoimmune diseases [35, 37]. A study analyzing various *H. pylori* antigens in 139 MS patients using enzyme-linked immunosorbent assay (ELISA) and immunoblotting reported that anti-VacA antibody was significantly more frequent in secondary progressive MS compared to controls, and some antigens such as anti-p26 have significant correlation with the EDSS score or the number of relapses [36]. However, it is still unclear whether the reactivity of *H. pylori* antibody for individual *H. pylori* antigens is pathologically associated with MS.

37.3.4 Guillain–Barré Syndrome

Guillain–Barré syndrome (GBS) is an acute autoimmune neuropathy characterized by progressive demyelization of peripheral nerves, usually triggered by infection. It was not initially assumed that *H. pylori* infection would cause acute demyelinating diseases, but after epidemiological studies, a significantly increased number of *H. pylori* infections were observed in patients with GBS compared to individuals without the disease [7] (Table 37.1). In a study including 13 of GBS patients, the *H. pylori* infection rate was higher in GBS patients than controls, and high anti-*H. pylori* IgG antibody levels were correlated with advanced clinical status [38]. In a study including 15 of GBS patients published in 2010, *H. pylori* antibody titers of serum and cerebrospinal fluid (CSF) were significantly higher in GBS patients than in the controls [39]. A meta-analysis including six studies published in 2020 reported that anti-*H. pylori* IgG were significantly more prevalent in GBS patients compared to controls, in both CSF (OR 42.45; 95% CI, 9.66–186.56; $Pz < 0.00001$) and serum (OR 2.31; 95% CI, 1.30–4.11; $Pz = 0.004$), suggesting that *H. pylori* infection may be involved in the pathophysiology of GBS [40].

Molecular mimicry of *H. pylori* has been proposed as a mechanism by which *H. pylori* may be involved in pathogenesis of GBS. *H. pylori* has a similar molecular structure of lipopolysaccharides to *Camphylobacter jejuni*, a representative strain that causes GBS, which can cause autoimmune sequelae [41]. In addition, because the molecular structures of VacA are similar to human adenosine triphosphate (ATP), there is a hypothesis that VacA can attach to schwann cells causing demyelization [42].

37.3.5 Ischemic Stroke

Ischemic stroke is a disease caused by carotid or cerebral vessel occlusion. Previous studies have suggested a strong association between *H. pylori* infection and ischemic stroke (Table 37.1). This association was emphasized in CagA-positive *H. pylori* strains. In a prospective population-based study of 684 patients published in 2003, the absolute values and changes of common carotid artery intima-media thickness (IMT) showed significant enhancement in CagA-seropositive subjects, but not in CagA-seronegative subjects for 5 years follow-up [43]. A study involving 185 patients reported that CagA-positive patients showed significantly higher IMT compared to CagA-negative patients (OR 2.36; 95% CI, 1.57–3.54; $p = 0.0001$) and poorer initial outcomes [44]. In a multicenter study published in 2008 comparing 106 ischemic stroke patients with 106 matched controls, CagA positivity was associated to a higher risk of atherosclerotic stroke (adjusted OR 2.69; 95% CI, 1.37–5.30) [45]. Most meta-analyses have reported similar results. A meta-analysis including 13 case-control studies with 4041 participants published in 2012 concluded that the positive in anti-*H. pylori* IgG, anti-CagA IgG, and 13^{C}-urea breath tests was significantly associated with an increased risk of ischemic stroke. In addition, this study reported that the positive anti-Cag A IgG was more effective for predication of ischemic stroke risk (OR 1.60; 95% CI, 1.21–2.11, $p = 0.001$) [46]. A recent meta-analysis reviewing 30 studies covering 273,135 patients published in 2021 showed that the positive anti-*H. pylori* IgG, anti-Cag A, and C-urea breath tests were significantly associated with an increased risk of stroke, respectively (OR 1.43; 95% CI, 1.25–1.46, OR 1.77; 95% CI, 1.25–2.49, and OR 2.21; 95% CI, 1.33–3.66), suggesting that *H. pylori* infection is significantly associated with increased risk of ischemic stroke [47].

The effect of *H. pylori* infection on ischemic stroke has suggested that cytotoxins and cytokines induced by *H. pylori* infection may interact with atherosclerotic plaques via local inflammatory reactions, including immune-mediated vascular damage, direct bacterial invasion to atherosclerosis plaques, hyperhomocysteinemia, and platelet aggregation [48, 49]. In particular, CagA strains have been considered to have more contribution to plaque instability and risk of ischemic stroke compared to CagA-negative strains, because they elicit a stronger and persistent low-grade immunoinflammatory burden [50]. A cross-mimicry between CagA protein and antigens of the endothelial wall of cerebral arteries has been demonstrated, which has been postulated to promote the atherosclerotic process by inducing endothelial damage [51].

37.3.6 Migraine

Some studies have reported a correlation between migraine and *H. pylori* infection. A case-control study including 103 migraine patients published in 2000 reported that there was no difference between the two groups [52]. In another case-control study of 175 migraine patients published in 2000, there was also no difference in prevalence between the two groups; however, among *H. pylori*-infected migraine patients, patients with aura showed significantly more CagA-positive strains than those without aura or controls (41% vs. 19%, $p < 0.01$ and 41% vs. 17%, $p < 0.01$) [53]. A meta-analysis including five case-control studies published in 2014 reported that the *H. pylori* infection rate was significantly higher in migraine patients than controls (44.97% vs. 33.26%, OR 1.92; 95% CI, 1.05–3.51; $p = 0.001$) [54]. In subgroup analysis, it was still statistically significant in Asians, but not statistically significant in Europeans (OR 3.48; 95% CI, 2.09–5.81; $p = 0.000$ and OR 1.19; 95% CI, 0.86–1.65; $p = 0.288$) [54]. A recent retrospective study including 526 migraine patients has reported that an incidence of white matter lesions (WML), which was considered as main observed pathological features in migraine, was significantly higher in *H. pylori*-positive patients compared to *H. pylori*-negative patients (OR 2.562; 95% CI, 1.784–3.680) [55].

The probable pathogenic role of *H. pylori* in migraine has been suggested as the immune response and the release of vasoactive substances caused by *H. pylori* infection [53, 54]. However, most studies to date have been retrospectively designed and included varied control groups. Therefore, further studies will be needed to confirm the association between *H. pylori* infection and migraine.

37.4 *H. pylori* Eradication Effects on Neurologic Diseases

37.4.1 Parkinson's Disease

In PD, *H. pylori* infection has been reported to be associated not only with the onset, but also with disease severity and drug response. The mechanism of how *H. pylori* may affect the drug response of levodopa, the main treatment drug for PD, remains unclear. Some hypotheses explain that duodenitis caused by *H. pylori* may delay gastric emptying time and consequently delay levodopa transfer to the duodenum which is the main absorption site of levodopa. In addition, *H. pylori* might indirectly interrupt levodopa absorption, by producing urease, thus raising the gastric pH and possibly affecting the pH-sensitive levodopa solubility [14]. Several studies reported that *H. pylori* eradication ameliorated the bioavailability of levodopa, resulting in improvement of the clinical response. A prospective study including 48 patients with PD published in 2017 showed that the *H. pylori* eradication group showed significant improvement in symptom after 1 year compared to baseline (18.3 ± 8.38 vs. 25.9 ± 8.37; $p = 0.007$) [82]. In a prospective study including 40 patients with PD published in 2021, *H. pylori* eradication group showed a significant decrease in daily 'off' time in which the effect of the drug is reduced (4.0 vs. 4.7 h; $p = 0.040$) and an increase in daily 'on' time in which the drug is effective (11.8 vs. 10.9 h; $p = 0.009$) compared to failed group. In addtion, this study showed that *H. pylori* eradication leads to significant clinical improvement in the symptoms of PD with total wearing-off score and gastrointestinal symptom score [83]. A recent meta-analysis including 13 studies published in 2022 showed that *H. pylori* infection was associated with worse motor symptoms, higher drug dosing, and worse drug response in patients with PD [14]. In this study, PD patients with *H. pylori* infection showed significantly lower UPDRS motor scores (SMD 0.266; 95% CI, 0.065–0.467; $p = 0.009$), higher levodopa equivalent daily dose (LEDD) (SMD 0.178; 95% CI, 0.004–0.353; $p = 0.046$), longer time to achieve 'on' state (SMD 0.778; 95% CI, 0.337–1.220; $p = 0.001$) and shorter duration of 'on' state (SMD −0.539; 95% CI, −0.801 to −0.227, $p = 0.001$) compared to those without *H. pylori* infection [14]. These results support the consideration of *H. pylori* eradication in PD patients.

37.4.2 Alzheimer's Disease

Several studies have suggested that eradication of *H. pylori* improved the survival rate and cognitive impairment parameters in AD. A study published in 2009 including 50 AD patients showed that the parameters of cognitive and functional status were improved in *H. pylori* eradication group at the 2-year clinical endpoint, but not in failed group ($p < 0.001$, $p = 0.049$, and $p < 0.001$; in Mini Mental State Examination, Cambridge Cognitive Examination for the Elderly, and Functional Rating Scale for Symptoms of Dementia, respectively) [84]. An observational study of 42 patients with AD published in 2010 reported that the survival rate was significantly higher in the eradication group than non-eradication group (HR 0.287; 95% CI, 0.114–0.725, $p = 0.008$) during the 5-year follow-up [85]. A large population-based study including 30,142 AD patients using the insurance of National Health Insurance of Taiwan published in 2013, although not modified by comorbidities, reported that *H. pylori* eradication was associated with a decreased risk of AD progression as compared to no eradication patients (OR 0.35; 95% CI, 0.23–0.52) [86]. However, most of the studies are only observational studies, and there are numerous confounding factors that affect the course of AD. Therefore, further studies will be needed to identify the beneficial role of *H. pylori* eradication in AD.

37.4.3 Other Diseases

Few clinical studies have reported the effectiveness of *H. pylori* eradication in other neurological diseases. A study including 48 patients with clinically isolated syndrome suggestive of MS reported that *H. pylori* eradication group showed significantly lower number of second episode and more improved symptoms at 2-year clinical endpoint [87]. A randomized, double blind, controlled study on migraine has reported that *H. pylori* eradication group showed a significant improvement in symptoms, but not in control group [88]. There is no study regarding the effects of *H. pylori* eradication on ischemic stroke; however, some authors speculated that *H. pylori* eradication may have a positive effect on the outcome of ischemic stroke because some previous studies have reported that the parameters related to atherosclerosis were significantly improved after *H. pylori* eradication. However, the evidence for the effect of *H. pylori* eradication on neurologic diseases is very much lacking.

37.5 Conclusions

H. pylori infection may affect the neurologic diseases via the MGBA as inducing inflammatory process, gut microbiome dysbiosis, and vitamin B12 deficiency. So far published studies have reported an association between *H. pylori* and various neurologic diseases such as PD, AD, MS, GBS, and ischemic stroke. However, there are limitations in that the study population and study methods are heterogeneous for each study, the number of study subjects is small due to the rarity of disease, and there are a lot of confounding factors. To clarify the causal relationship between *H. pylori* infection and neurologic diseases, and by extend, to identify the benefits of eradication in neurological disorders, further well-designed studies will be needed.

References

1. Gorlé N, Bauwens E, Haesebrouck F, Smet A, Vandenbroucke RE. *Helicobacter* and the potential role in neurological disorders: there is more than *Helicobacter pylori*. Front Immunol. 2021;11:584165.
2. Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, et al. The microbiota-gut-brain axis. Physiol Rev. 2019;99:1877–2013.
3. Liu L, Huh JR, Shah K. Microbiota and the gut-brain-axis: implications for new therapeutic design in the CNS. EBioMedicine. 2022;77:103908.
4. Spichak S, Bastiaanssen TFS, Berding K, Vlckova K, Clarke G, Dinan TG, et al. Mining microbes for mental health: determining the role of microbial metabolic pathways in human brain health and disease. Neurosci Biobehav Rev. 2021;125:698–761.
5. Tran VTA, Lee LP, Cho H. Neuroinflammation in neurodegeneration via microbial infections. Front Immunol. 2022;13:907804.
6. Budzyński J, Kłopocka M. Brain-gut axis in the pathogenesis of *Helicobacter pylori* infection. World J Gastroenterol. 2014;20:5212–25.
7. Baj J, Forma A, Flieger W, Morawska I, Michalski A, Buszewicz G, et al. *Helicobacter pylori* infection and extragastric diseases-A focus on the central nervous system. Cell. 2021;10:2191.
8. Alvarez-Arellano L, Maldonado-Bernal C. *Helicobacter pylori* and neurological diseases: married by the laws of inflammation. World J Gastrointest Pathophysiol. 2014;5:400–4.
9. Chen Y, Xu J, Chen Y. Regulation of neurotransmitters by the gut microbiota and effects on cognition in neurological disorders. Nutrients. 2021;13:2099.
10. Altschuler E. Gastric *Helicobacter pylori* infection as a cause of idiopathic Parkinson disease and non-arteric anterior optic ischemic neuropathy. Med Hypotheses. 1996;47:413–4.
11. Shen X, Yang H, Wu Y, Zhang D, Jiang H. Meta-analysis: association of *Helicobacter pylori* infection with Parkinson's diseases. Helicobacter. 2017;22:e12398.
12. Dardiotis E, Tsouris Z, Mentis AA, Siokas V, Michalopoulou A, Sokratous M, et al. *H. pylori* and Parkinson's disease: meta-analyses including clinical severity. Clin Neurol Neurosurg. 2018;175:16–24.
13. Huang HK, Wang JH, Lei WY, Chen CL, Chang CY, Liou LS. *Helicobacter pylori* infection is associated with an increased risk of Parkinson's disease: a population-based retrospective cohort study. Parkinsonism Relat Disord. 2018;47:26–31.
14. Zhong R, Chen Q, Zhang X, Li M, Lin W. *Helicobacter pylori* infection is associated with a poor response to levodopa in patients with Parkinson's disease: a systematic review and meta-analysis. J Neurol. 2022;269:703–11.
15. Béraud D, Maguire-Zeiss KA. Misfolded α-synuclein and Toll-like receptors: therapeutic targets for Parkinson's disease. Parkinsonism Relat Disord. 2012;18 (Suppl 1):S17–20.
16. Rodriguez MC, Obeso JA, Olanow CW. Subthalamic nucleus-mediated excitotoxicity in Parkinson's disease: a target for neuroprotection. Ann Neurol. 1998;44:S175–88.
17. Malaguarnera M, Bella R, Alagona G, Ferri R, Carnemolla A, Pennisi G. *Helicobacter pylori* and Alzheimer's disease: a possible link. Eur J Intern Med. 2004;15:381–6.
18. Kountouras J, Tsolaki M, Gavalas E, Boziki M, Zavos C, Karatzoglou P, et al. Relationship between *Helicobacter pylori* infection and Alzheimer disease. Neurology. 2006;66:938–40.
19. Shiota S, Murakami K, Yoshiiwa A, Yamamoto K, Ohno S, Kuroda A, et al. The relationship between *Helicobacter pylori* infection and Alzheimer's disease in Japan. J Neurol. 2011;258:1460–3.
20. Huang WS, Yang TY, Shen WC, Lin CL, Lin MC, Kao CH. Association between *Helicobacter pylori* infection and dementia. J Clin Neurosci. 2014;21:1355–8.
21. Fani L, Wolters FJ, Ikram MK, Bruno MJ, Hofman A, Koudstaal PJ, et al. *Helicobacter pylori* and the risk of dementia: a population-based study. Alzheimers Dement. 2018;14:1377–82.
22. Beydoun MA, Beydoun HA, Elbejjani M, Dore GA, Zonderman AB. *Helicobacter pylori* seropositivity and its association with incident all-cause and Alzheimer's disease dementia in large national surveys. Alzheimers Dement. 2018;14:1148–58.
23. Liu NY, Sun JH, Jiang XF, Li H. *Helicobacter pylori* infection and risk for developing dementia: an evidence-based meta-analysis of case-control and cohort studies. Aging (Albany NY). 2021;13:22571–87.
24. Kountouras J, Gavalas E, Zavos C, Stergiopoulos C, Chatzopoulos D, Kapetanakis N, et al. Alzheimer's disease and *Helicobacter pylori* infection: defective immune regulation and apoptosis as proposed common links. Med Hypotheses. 2007;68:378–88.
25. Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med. 2002;346:476–83.
26. Roubaud-Baudron C, Krolak-Salmon P, Quadrio I, Mégraud F, Salles N. Impact of chronic *Helicobacter pylori* infection on Alzheimer's disease: preliminary results. Neurobiol Aging. 2012;33:1009.e11–9.
27. Papadopoulos MC, Verkman AS. Aquaporin 4 and neuromyelitis optica. Lancet Neurol. 2012;11:535–44.
28. Li W, Minohara M, Su JJ, Matsuoka T, Osoegawa M, Ishizu T, et al. *Helicobacter pylori* infection is a potential protective factor against conventional

multiple sclerosis in the Japanese population. J Neuroimmunol. 2007;184:227–31.

29. Li W, Minohara M, Piao H, Matsushita T, Masaki K, Matsuoka T, et al. Association of anti-*Helicobacter pylori* neutrophil-activating protein antibody response with anti-aquaporin-4 autoimmunity in Japanese patients with multiple sclerosis and neuromyelitis optica. Mult Scler. 2009;15:1411–21.
30. Long Y, Gao C, Qiu W, Hu X, Shu Y, Peng F, et al. *Helicobacter pylori* infection in Neuromyelitis Optica and Multiple Sclerosis. Neuroimmunomodulation. 2013;20:107–12.
31. Gavalas E, Kountouras J, Boziki M, Zavos C, Polyzos SA, Vlachaki E, et al. Relationship between *Helicobacter pylori* infection and multiple sclerosis. Ann Gastroenterol. 2015;28:353–6.
32. Arjmandi D, Abdollahi A, Ardekani A, Razavian I, Razavian E, Sartip B, et al. *Helicobacter pylori* infection and risk of multiple sclerosis: an updated meta-analysis. Helicobacter. 2022;27:e12927.
33. Rook GAW. Hygiene hypothesis and autoimmune diseases. Clin Rev Allergy Immunol. 2012;42:5–15.
34. Thompson AJ, Baranzini SE, Geurts J, Hemmer B, Ciccarelli O. Multiple sclerosis. Lancet. 2018;391:1622–36.
35. Gerges SE, Alosh TK, Khalil SH, El Din MMW. Relevance of *Helicobacter pylori* infection in Egyptian multiple sclerosis patients. Egypt J Neurol Psychiatr Neurosurg. 2018;54:41.
36. Efthymiou G, Dardiotis E, Liaskos C, Marou E, Tsimourtou V, Rigopoulou EI, et al. Immune responses against *Helicobacter pylori*-specific antigens differentiate relapsing remitting from secondary progressive multiple sclerosis. Sci Rep. 2017;7:7929.
37. Efthymiou G, Dardiotis E, Liaskos C, Marou E, Tsimourtou V, Scheper T, et al. Anti-hsp60 antibody responses based on *Helicobacter pylori* in patients with multiple sclerosis: (ir)Relevance to disease pathogenesis. J Neuroimmunol. 2016;298:19–23.
38. Kountouras J, Deretzi G, Zavos C, Karatzoglou P, Touloumis L, Nicolaides T, et al. Association between *Helicobacter pylori* infection and acute inflammatory demyelinating polyradiculoneuropathy. Eur J Neurol. 2005;12:139–43.
39. Ghabaee M, Ghanbarian D, Brujeni GN, Bokaei S, Siavoshi F, Gharibzadeh S. Could *Helicobacter pylori* play an important role in axonal type of Guillain-Barré syndrome pathogenesis? Clin Neurol Neurosurg. 2010;112:193–8.
40. Dardiotis E, Sokratous M, Tsouris Z, Siokas V, Mentis AA, Aloizou AM, et al. Association between *Helicobacter pylori* infection and Guillain-Barré Syndrome: a meta-analysis. Eur J Clin Investig. 2020;50:e13218.
41. Moran AP, Prendergast MM. Molecular mimicry in *Campylobacter jejuni* and *Helicobacter pylori* lipopolysaccharides: contribution of gastrointestinal infections to autoimmunity. J Autoimmun. 2001;16:241–56.
42. Chiba S, Sugiyama T, Yonekura K, Tanaka S, Matsumoto H, Fujii N, et al. An antibody to VacA of *Helicobacter pylori* in cerebrospinal fluid from patients with Guillain-Barré syndrome. J Neurol Neurosurg Psychiatry. 2002;73:76–8.
43. Mayr M, Kiechl S, Mendall MA, Willeit J, Wick G, Xu Q. Increased risk of atherosclerosis is confined to CagA-positive *Helicobacter pylori* strains: prospective results from the Bruneck study. Stroke. 2003;34:610–5.
44. Diomedi M, Pietroiusti A, Silvestrini M, Rizzato B, Cupini LM, Ferrante F, et al. CagA-positive *Helicobacter pylori* strains may influence the natural history of atherosclerotic stroke. Neurology. 2004;63:800–4.
45. De Bastiani R, Gabrielli M, Ubaldi E, Benedetto E, Sanna G, Cottone C, et al. High prevalence of Cag-A positive *H. pylori* strains in ischemic stroke: a primary care multicenter study. Helicobacter. 2008;13:274–7.
46. Wang ZW, Li Y, Huang LY, Guan QK, Xu DW, Zhou WK, et al. *Helicobacter pylori* infection contributes to high risk of ischemic stroke: evidence from a meta-analysis. J Neurol. 2012;259:2527–37.
47. Doheim MF, Altaweel AA, Elgendy MG, Elshanbary AA, Dibas M, Ali A, et al. Association between *Helicobacter pylori* infection and stroke: a meta-analysis of 273,135 patients. J Neurol. 2021;268:3238–48.
48. Sawayama Y, Ariyama I, Hamada M, Otaguro S, Machi T, Taira Y, et al. Association between chronic *Helicobacter pylori* infection and acute ischemic stroke: fukuoka Harasanshin Atherosclerosis Trial (FHAT). Atherosclerosis. 2005;178:303–9.
49. Chmiela M, Gajewski A, Rudnicka K. *Helicobacter pylori* vs coronary heart disease—searching for connections. World J Cardiol. 2015;7:187–203.
50. Diomedi M, Stanzione P, Sallustio F, Leone G, Renna A, Misaggi G, et al. Cytotoxin-associated gene-A-positive *Helicobacter pylori* strains infection increases the risk of recurrent atherosclerotic stroke. Helicobacter. 2008;13:525–31.
51. Gabrielli M, Pola P, Gasbarrini A. *Helicobacter pylori*, CagA-positive strains, and ischemic stroke. Stroke. 2002;33:1453–4.
52. Pinessi L, Savi L, Pellicano R, Rainero I, Valfrè W, Gentile S, et al. Chronic *Helicobacter pylori* infection and migraine: a case-control study. Headache. 2000;40:836–9.
53. Gasbarrini A, Gabrielli M, Fiore G, Candelli M, Bartolozzi F, De Luca A, et al. Association between *Helicobacter pylori* cytotoxic type I CagA-positive strains and migraine with aura. Cephalalgia. 2000;20:561–5.
54. Su J, Zhou XY, Zhang GX. Association between *Helicobacter pylori* infection and migraine: a meta-analysis. World J Gastroenterol. 2014;20:14965–72.
55. Öcal S, Öcal R, Suna N. Relationship between *Helicobacter pylori* infection and white matter lesions in patients with migraine. BMC Neurol. 2022;22:187.

56. Charlett A, Dobbs RJ, Dobbs SM, Weller C, Brady P, Peterson DW. Parkinsonism: siblings share *Helicobacter pylori* seropositivity and facets of syndrome. Acta Neurol Scand. 1999;99:26–35.
57. Dobbs RJ, Charlett A, Dobbs SM, Weller C, Peterson DW. Parkinsonism: differential age-trend in *Helicobacter pylori* antibody. Aliment Pharmacol Ther. 2000;14:1199–205.
58. Nielsen HH, Qiu J, Friis S, Wermuth L, Ritz B. Treatment for *Helicobacter pylori* infection and risk of Parkinson's disease in Denmark. Eur J Neurol. 2012;19:864–9.
59. Nägga K, Rajani R, Mårdh E, Borch K, Mårdh S, Marcusson J. Cobalamin, folate, methylmalonic acid, homocysteine, and gastritis markers in dementia. Dement Geriatr Cogn Disord. 2003;16:269–75.
60. Tsolaki F, Kountouras J, Topouzis F, Tsolaki M. *Helicobacter pylori* infection, dementia and primary open-angle glaucoma: are they connected? BMC Ophthalmol. 2015;15:24.
61. Yoshimura S, Isobe N, Matsushita T, Yonekawa T, Masaki K, Sato S, et al. Distinct genetic and infectious profiles in Japanese neuromyelitis optica patients according to anti-aquaporin 4 antibody status. J Neurol Neurosurg Psychiatry. 2013;84:29–34.
62. Mohebi N, Mamarabadi M, Moghaddasi M. Relation of *Helicobacter pylori* infection and multiple sclerosis in Iranian patients. Neurol Int. 2013;5:31–3.
63. Cook KW, Crooks J, Hussain K, O'Brien K, Braitch M, Kareem H, et al. *Helicobacter pylori* infection reduces disease severity in an experimental model of multiple sclerosis. Front Microbiol. 2015;6:52.
64. Malli C, Pandit L, D'Cunha A, Mustafa S. Environmental factors related to multiple sclerosis in Indian population. PLoS One. 2015;10:e0124064.
65. Pedrini MJ, Seewann A, Bennett KA, Wood AJ, James I, Burton J, et al. *Helicobacter pylori* infection as a protective factor against multiple sclerosis risk in females. J Neurol Neurosurg Psychiatry. 2015;86:603–7.
66. Kiani S, Vakilian A, Kamiab Z, Shamsizadeh A. Correlation of dietary intake and *Helicobacter pylori* infection with multiple sclerosis, a case-control study in Rafsanjan, Iran, 2017-18. Qatar Med J. 2020;2020:45.
67. Mirmosayyeb O, Barzegar M, Nehzat N, Najdaghi S, Ansari B, Shaygannejad V. Association of *Helicobacter pylori* with multiple sclerosis: protective or risk factor? Curr J Neurol. 2020;19:59–66.
68. Annunziata P, Figura N, Galli R, Mugnaini F, Lenzi C. Association of anti-GM1 antibodies but not of anti-cytomegalovirus, Campylobacter jejuni and *Helicobacter pylori* IgG, with a poor outcome in Guillain–Barré syndrome. J Neurol Sci. 2003;213:55–60.
69. Chiba S, Sugiyama T, Yonekura K, Tanaka S, Matsumoto H, Fujii N, et al. An antibody to VacA of *Helicobacter pylori* in the CSF of patients with Miller-Fisher syndrome. Neurology. 2004;63:2184.
70. Markus HS, Mendall MA. *Helicobacter pylori* infection: a risk factor for ischaemic cerebrovascular disease and carotid atheroma. J Neurol Neurosurg Psychiatry. 1998;64:104–7.
71. Grau AJ, Buggle F, Lichy C, Brandt T, Becher H, Rudi J. *Helicobacter pylori* infection as an independent risk factor for cerebral ischemia of atherothrombotic origin. J Neurol Sci. 2001;186:1–5.
72. Heuschmann PU, Neureiter D, Gesslein M, Craiovan B, Maass M, Faller G, et al. Association between infection with *Helicobacter pylori* and Chlamydia pneumoniae and risk of ischemic stroke subtypes: results from a population-based case-control study. Stroke. 2001;32:2253–8.
73. Ponzetto A, Marchet A, Pellicano R, Lovera N, Chianale G, Nobili M, et al. Association of *Helicobacter pylori* infection with ischemic stroke of non-cardiac origin: the BAT.MA.N. project study. Hepato-Gastroenterology. 2002;49:631–4.
74. Pietroiusti A, Diomedi M, Silvestrini M, Cupini LM, Luzzi I, Gomez-Miguel MJ, et al. Cytotoxin-associated gene-A—positive *Helicobacter pylori* strains are associated with atherosclerotic stroke. Circulation. 2002;106:580–4.
75. Moayyedi P, Carter AM, Braunholtz D, Catto AJ. *Helicobacter pylori* infection in subjects with acute ischaemic stroke. Dig Liver Dis. 2003;35:16–9.
76. Gabrielli M, Santoliquido A, Cremonini F, Cicconi V, Candelli M, Serricchio M, et al. CagA-positive cytotoxic *H. pylori* strains as a link between plaque instability and atherosclerotic stroke. Eur Heart J. 2004;25:64–8.
77. Preusch MR, Grau AJ, Buggle F, Lichy C, Bartel J, Black C, et al. Association between cerebral ischemia and cytotoxin-associated gene-A-bearing strains of *Helicobacter pylori*. Stroke. 2004;35:1800–4.
78. Ashtari F, Shayegannejad V, Saberi A, Rabiee E. Relationship between *Helicobacter pylori* immunoglobulin G antibody and thrombotic ischemic stroke. Acta Med Iran. 2008;46:303.
79. Elkind MS, Ramakrishnan P, Moon YP, Boden-Albala B, Liu KM, Spitalnik SL, et al. Infectious burden and risk of stroke: the northern Manhattan study. Arch Neurol. 2010;67:33–8.
80. Schöttker B, Adamu MA, Weck MN, Müller H, Brenner H. *Helicobacter pylori* infection, chronic atrophic gastritis and major cardiovascular events: a population-based cohort study. Atherosclerosis. 2012;220:569–74.
81. Huang WS, Tseng CH, Lin CL, Tsai CH, Kao CH. *Helicobacter pylori* infection increases subsequent ischemic stroke risk: a nationwide population-based retrospective cohort study. QJM. 2014;107:969–75.
82. Liu H, Su W, Li S, Du W, Ma X, Jin Y, et al. Eradication of *Helicobacter pylori* infection might improve clinical status of patients with Parkinson's disease, especially on bradykinesia. Clin Neurol Neurosurg. 2017;160:101–4.
83. Lolekha P, Sriphanom T, Vilaichone RK. *Helicobacter pylori* eradication improves motor fluctuations

in advanced Parkinson's disease patients: a prospective cohort study (HP-PD trial). PLoS One. 2021;16:e0251042.

84. Kountouras J, Boziki M, Gavalas E, Zavos C, Grigoriadis N, Deretzi G, et al. Eradication of *Helicobacter pylori* may be beneficial in the management of Alzheimer's disease. J Neurol. 2009;256:758–67.
85. Kountouras J, Boziki M, Gavalas E, Zavos C, Deretzi G, Chatzigeorgiou S, et al. Five-year survival after *Helicobacter pylori* eradication in Alzheimer disease patients. Cogn Behav Neurol. 2010;23:199–204.
86. Chang YP, Chiu GF, Kuo FC, Lai CL, Yang YH, Hu HM, et al. Eradication of *Helicobacter pylori* is associated with the progression of dementia: a population-based study. Gastroenterol Res Pract. 2013;2013:175729.
87. Deretzi G, Gavalas E, Boziki M, Tsiptsios D, Polyzos SA, Venizelos I, et al. Impact of *Helicobacter pylori* on multiple sclerosis-related clinically isolated syndrome. Acta Neurol Scand. 2016;133:268–75.
88. Faraji F, Zarinfar N, Zanjani AT, Morteza A. The effect of *Helicobacter pylori* eradication on migraine: a randomized, double blind, controlled trial. Pain Physician. 2012;15:495–8.

Part VI

Antibiotic Resistance

38

Synopsis of Antimicrobial Resistance

Francis Mégraud

Abstract

Helicobacter pylori resistance to antibiotics is the main cause of failure of the eradication treatments. The essential mechanism of resistance acquisition is the occurrence of point mutations in genes important for the antimicrobial activity, while efflux pumps have also been described. Resistance to macrolides is steadily increasing around the world and is clinically significant. Beside the standard methods, it can be detected in a few hours by molecular methods, especially real-time PCR followed by a melting curve analysis. Resistance to fluoroquinolones is also important for the same reasons, and some molecular methods can also be applied. Metronidazole resistance is very common, but it can be overcome in vivo. Its in vitro detection lacks reproducibility, and no molecular method is currently available. Resistance to the other antibiotics of potential interest such as for amoxicillin, tetracycline, and rifamycin is seldom found.

Keywords

Point mutations · Clarithromycin · Real-time PCR · Prevalence

38.1 Introduction

The discovery of *Helicobacter pylori* (*H. pylori*) was a breakthrough in the field of gastroenterology [1]. Diseases such as peptic ulcer disease for which there was no efficient treatment could suddenly be cured. It is now recognized that most gastroduodenal diseases are indeed infectious diseases and so can be treated with antibiotics. However, a single antibiotic was not sufficient, and in the beginning of the 1990s, a treatment using two antibiotics was proposed, to which an antisecretory drug, as an adjuvant treatment to increase the pH, had to be added [2]. This treatment was successful for almost two decades, but unfortunately *H. pylori* became progressively resistant to some of the antibiotics used, and nowadays, it is no longer a valid option in many regions of the world [3].

In this chapter, we shall review the mechanisms of antibiotic resistance, its clinical consequences, the methods that can be used to detect this resistance, and the current prevalence.

38.2 Mechanisms of Resistance to Antibiotics

Bacteria can become resistant to antibiotics by two major mechanisms: (1) acquisition of mobile genetic elements which confer resistance or (2) point mutations which modify the structure of the target [4].

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38.2.1 Acquisition of Mobile Genetic Elements

Indeed, this mechanism is the main one in the microbial world. Bacteria can exchange part of their DNA by different mechanisms like conjugation and transduction. By conjugation, the elements are named plasmids and can be transferred from one bacterium (donor) to another (recipient). The plasmid exchange is encoded by the plasmid itself. It is transferred horizontally. It occurs like an outbreak, and most of the bacteria become resistant over a short time lapse. Other mobile genetic elements are the transposons and integrons. Mainly concerning Gram-positive bacteria, the transfer requires a bacteriophage. This is the so-called transduction.

In addition, DNA fragments issued from dead bacteria can also be present in the environment. At a specific moment of the bacterial cycle, these DNA fragments can be integrated into the bacteria. This is the so-called transformation. In all cases, the DNA fragment is able to integrate to the bacterial chromosome and transfer its genes which can concern resistance to antibiotics. Fortunately, this horizontal transmission of resistance is not observed in *H. pylori*.

38.2.2 Resistance Acquired by Point Mutation

Point mutations occur relatively often in bacterial DNA. They correspond to errors in the DNA synthesis. Many point mutations in a gene sequence do not have visible consequences, but others can alter all of the physiological aspects of the bacterium, including antibiotic resistance. Point mutation is the essential mechanism of acquisition of resistance in *H. pylori* and concerns most of the antibiotics used to treat this infection, albeit at very different rates. *H. pylori* shares this particularity with another important bacterium: *Mycobacterium tuberculosis*. Indeed, the point mutation modifies critical sites in target molecules for the antibiotic's activity. The list is presented in Table 38.1. In contrast to the resistance acquired by mobile genetic elements, the resistance acquired by point mutation is transmitted vertically and the proportion of resistant bacteria increases slowly. This explains why a heteroresistance is observed when only some elements of the population are detected as resistant. However, when there is antibiotic pressure, the selection occurs drastically. It is also dependent on the impact of the mutation on the bacterial fitness, especially its ability to replicate. If the mutation has such an impact, then when the selective pressure stops, the bacteria harboring the mutation will progressively disappear from the population, and the wild type without this limitation will only remain.

Table 38.1 Genes concerned by point mutations or other genetic events leading to antibiotic resistance in *H. pylori*

Antibiotic group	Genes concerned
Macrolides	*rrn23S*
Metronidazole	*rdxA*, *frxA*
Quinolones	*gyrA*
Rifamycins	*rpoB*
Amoxicillin	*pbp-1*
Tetracyclines	*rrn16S*

38.2.3 Efflux Pumps and *H. pylori* Resistance

To maintain cellular homeostasis, some bacteria can actively expel harmful molecules outside of the cell. This mechanism was first described for tetracycline in the 1980s. Hyperproduction of the so-called efflux pumps results in an increase in minimal inhibitory concentration (MIC) values. This mechanism is not common in *H. pylori,* but can occur [5, 6].

38.3 Resistance Observed in *H. pylori* and Its Consequences

There are six antibiotic groups which can be used to treat *H. pylori* infection, but the problem of resistance is crucial for only two: macrolides (clarithromycin) and fluoroquinolones (levofloxacin), because of their common use and current high prevalence observed. A second group is composed of amoxicillin, tetracycline, and rifabutin because resistance is seldom found or the compound rarely used. Finally, 5-nitroimidazoles (metronidazole) must be considered separately because of their particularity.

38.3.1 Macrolides

The mechanisms of action of macrolides consist of binding to ribosomes at the level of the peptidyl transferase loop of the 23S rRNA gene. Point mutations occurring in this loop may change its conformation and limit the binding possibility [7]. The main positions described as inducing resistance are the transition A2142G, A2143G [7, 8], and the transversion A2142C [9]. There are also rare cases with A2142T. Other mutations have been described, but their impact on the MICs of the bacteria has not been definitely proven. It is interesting to note that all macrolides are concerned by the occurrence of these conformational changes. There is a cross-resistance between all of them. In terms of frequency, transition is by far the most common. The resulting MICs are diverse, and there is no agreement on the mutations which would lead to the highest MIC.

When the standard clarithromycin-based therapy is used, in case of resistance, only the second antibiotic (usually amoxicillin) remains active, but the doses and frequency of administration are not sufficient to obtain *H. pylori* eradication, and there is therefore a limited rate of success. Several meta-analyses have shown that when a strain is clarithromycin-susceptible, the rate of success is in the range of 90% (indeed there are other reasons for failure, e.g., bad compliance, insufficient acid reduction, high bacterial load, etc.), while if the strain is clarithromycin-resistant, the rate of success decreases to the range of 20% and even less if the second antibiotic is metronidazole and resistance to this drug is present.

38.3.2 Fluoroquinolones

The mechanism of action of fluoroquinolones consists of inhibiting the A subunit of the DNA gyrase encoded by the *gyrA* gene. There is a special part of the gene, namely, the quinolone resistance determining region (QRDR), where occurrence of point mutations leads to a lack of binding. There are merely two amino acids concerned in positions 87 and 91 [10, 11], but mutations at other positions can be found such as recently in Korea [12]. There is usually a cross-resistance between the various fluoroquinolones. However, new compounds such as sitafloxacin, used in the Far East, show much lower MICs in case of mutation than it is with levofloxacin and possibly concentrations achievable with the clinical dose used.

When the levofloxacin-amoxicillin-proton pump inhibitor (PPI) regimen is used, generally as a second-line treatment, there is a major impact of levofloxacin resistance.

38.3.3 Amoxicillin

As the other ß-lactams, amoxicillin acts by interfering with the peptidoglycan synthesis, by blocking transporters named penicillin-binding proteins (PBP). However, amoxicillin resistance is rare in *H. pylori*. It was first described in a patient having received multiple cures of this antibiotic for respiratory tract infections. The mechanism identified was a mutation occurring in the *pbp1A* gene, in particular, a Ser414 → Arg mutation leading to a PBP blockage; but, the resistance level was not very high [13]. Another mechanism described is the lack of a specific PBP, PBP-D [14]. By looking at results of prevalence of antimicrobial resistance, one can see in a few articles a relatively high prevalence of amoxicillin resistance. Such results must be considered with caution if they were not verified and the mechanisms explored. However, mutations other than those already described in the *pbp1* gene [15] or in other genes [16] could contribute to resistance.

38.3.4 Tetracyclines

The action mechanism of tetracyclines is an interference in protein synthesis by binding to the 30S ribosomal subunit. By three adjacent mutations, a nucleotide triplet can be changed (AGA926 to 928TTC) at the 16S rRNA gene level, interfering with binding to the h1 loop, the binding site of tetracyclines [17, 18]. The rarity of tetracycline resistance is due to the low probability to obtain three adjacent mutations. Single or dual mutations are logically more frequent leading to slightly increased MICs.

As stated before, another mechanism may be responsible for resistance in the absence of mutation, i.e., the efflux mechanism. These strains exhibit a decreased accumulation of tetracycline inside the cells. While all tetracyclines may inhibit *H. pylori*, the most active remains tetracycline HCl. It is this drug which is part of the active combination Pylera®, the three-in-one capsule now available to treat *H. pylori* infection.

38.3.5 Rifampin

The mechanism of action of the rifampin is an inhibition of the B subunit of the DNA-dependent RNA polymerase encoded by the *rpoB* gene. As with *M. tuberculosis*, mutations can occur in this gene at specific positions, mainly 524, 525, and 585 [19]. Rifampin resistance is relatively rare, because of the low selective pressure due to these antibiotics. Indeed, they are mainly used to treat tuberculosis, and *H. pylori* resistance has been essentially described in patients having received rifampin to eradicate *M. tuberculosis*. The main rifampin used to treat *H. pylori* infection is rifabutin, but there is a cross-resistance among this group of antibiotics.

38.3.6 5-Nitroimidazoles

5-Nitroimidazoles are mainly used to treat anaerobic infections, but they have shown their efficacy on the microaerophilic bacterium, i.e., *H. pylori*. The compound is a prodrug which must be reduced into a nitrosamine inside the bacterial cell to alter bacterial DNA. To achieve this reduction, there is primarily an oxygen-insensitive nitroreductase encoded by the *rdxA* gene, but a mutation in this gene can render the protein ineffective [20]. Another enzyme, flavodoxin oxidoreductase (FrxA), may also be involved in the reduction process [21]. A further mechanism of resistance, again the efflux system, could also play a role [6].

The most commonly used compound for *H. pylori* eradication is metronidazole, but tinidazole which has a better pharmacokinetic profile has also been used. The particularity of these compounds with regard to resistance is the poor correlation between in vitro and in vivo data, to which the problem of a poor reproducibility of in vitro data must be added [22]. This fact could be explained by the lack of control of the redox potential, which is important in the reduction of 5-nitroimidazoles. Furazolidone, a nitrofurane compound, can be used instead of metronidazole in countries where it is available, but may lead to adverse events.

As a general observation, it must also be said that *H. pylori* resistance may also be influenced by the status of the bacteria either planktonic or in biofilms, and it may come from the efflux pump genes which are more expressed when the bacteria are forming biofilms [23].

38.4 Methods to Detect *H. pylori* Resistance

The standard method to determine *H. pylori* susceptibility or resistance to antimicrobial compounds is the phenotypic method, i.e., the determination of the MICs against the bacterium. However, given the mechanism involved, it was relatively easy to design molecular methods to look for mutations, and this approach is now more common especially for the main antibiotic used, clarithromycin.

38.4.1 Phenotypic Methods

The current recommendation in Europe (EUCAST) is to perform an agar diffusion method with E-test. E-test is especially adapted to slow-growing bacteria such as *H. pylori*, and a good correlation has been found with the dilution method except for metronidazole as indicated before [22, 24]. The reference method for clarithromycin susceptibility testing remains agar dilution as proposed by the Clinical Laboratory Standard Institute (CLSI) in the USA. A broth detection method can also be used, but a broth supplementation must be added to allow a good growth of *H. pylori* [25]. Agar diffusion using disks was not recommended. Nevertheless, it can be used as a screening method because it is simple and cheap, before using E-test in case of doubtful results. A recent study showed >90% agreement with the E-test for clarithromycin, levofloxacin, and metronidazole [26].

38.4.2 Genotypic Methods

A large number of genotypic methods have been designed especially to detect the mutations associated with clarithromycin resistance, as they are indeed the main reason for failure of the clarithromycin-based triple therapy which was recommended worldwide beginning at the end of the last century.

These numerous methods have been recently reviewed [27]. The most commonly employed method is a real-time PCR which is able to detect, first, *H. pylori* and, if present, second, the mutation associated with macrolide resistance.

There are different variants of the method. One is to design *H. pylori*-specific primers on the 23S rRNA gene and then a biprobe (sensor probe and anchor probe) hybridizing close to each other in order to allow a transfer of energy, according to the fluorescence resonance energy transfer (FRET) principle. This method allows the amplification to be followed in real-time, and then a melting curve analysis (MCA) is performed. In the absence of a mutation, there is a perfect match of the nucleotides and the melting temperature is the highest, while if a mutation is present, there is a mismatch and the melting temperature is lower (e.g., 62 °C for the former and 58–53 °C for the latter, according to the type of mutation) [28]. The main advantage of this method is its rapidity, given that a result can be obtained within 2 h after obtention of a gastric biopsy. It also limits the possibility of contamination with amplicons because the reaction is performed in a closed tube. An adaptation of this method to microwell plates was developed in 2022. The reagent mix, prepared under the required conditions, can be bought quite cheaply. The LightCycler 4800 adapted to the FRET methodology must be used [29]. Other kits are also commercially available allowing a quick detection of *H. pylori* and the eventual mutation leading to clarithromycin resistance. There is also a tendency to use kits on apparati such us the BD Max™ [30] or the Smart Gene™ [31] systems which include the extraction and simplify the process. In addition, these methods have been used on stool specimens [32]. The main limitation in this environment is the possibility of false-negative results because the amount of *H. pylori* DNA is low in the stools and because inhibitors of the Taq polymerase from the diet can be present [33]. It is important to use a very effective method of DNA isolation. The FRET-MCA method was also applied to detect *H. pylori* resistance to fluoroquinolones using two biprobes [34].

Following the development of Next Generation Sequencing (NGS), this technique was applied for antimicrobial susceptibility testing. Hulten et al. [35] compared NGS performed on isolates targeting the genes involved in comparison to the phenotypic method based on antibiograms for the main antibiotics of interest to treat *H. pylori* infection. They obtained a very good agreement for clarithromycin and good for levofloxacin while it was only fair for metronidazole and amoxicillin. The lack of resistance precluded comparisons for tetracycline and rifabutin.

Interestingly, they also performed NGS on formalin-fixed paraffin-embedded gastric biopsies as well as on stools with the same results [36].

The problem of this method being the cost, it remains a research tool especially to better characterize the frequent cases of metronidazole and the rare cases of amoxicillin resistance. Its interest is very limited for tetracycline and rifabutin given the rarity of resistance, while for clarithromycin and to a lesser extent levofloxacin, the mutation sites are limited with the same positions favoring a detection by real-time PCR.

38.5 Prevalence of *H. pylori* Resistance

The relevance of *H. pylori* resistance depends on the prevalence observed. This prevalence is an evolving process which must be followed at successive time points [37].

Numerous studies have been performed. The problem is to test a population representative of the general population, while many studies are issued from referral centers for *H. pylori* treatment with a risk of including patients harboring strains which are not "primary" resistant. It is also important to test an important number of cases, in order to get small confidence intervals of the results. In Europe, the survey performed in 2008–2009 [38] was conducted again 10 years later (2018–2019) [39]. *H. pylori* isolates from 1211 patients were investigated in 24 centers from 18 European countries according to a standardized protocol. The global prevalence of primary clarithromycin resistance was 21.4% and 15.8% for levofloxacin resistance, i.e., a significant increase from 17.5% for the former while it was slightly increased from 14.1% for the latter. When compared to 10 years before, the same important variations between countries were found, the highest prevalence being in Central/Western and Southern Europe. A positive correlation with the antibiotic consumption in the community was also found for these two antibiotics (Fig. 38.1).

The status of *H. pylori* resistance in the United States has been explored in a systematic review and meta-analysis published in 2022 [40]. Nineteen studies including 2660 samples obtained between 2011 and 2021 were reviewed.

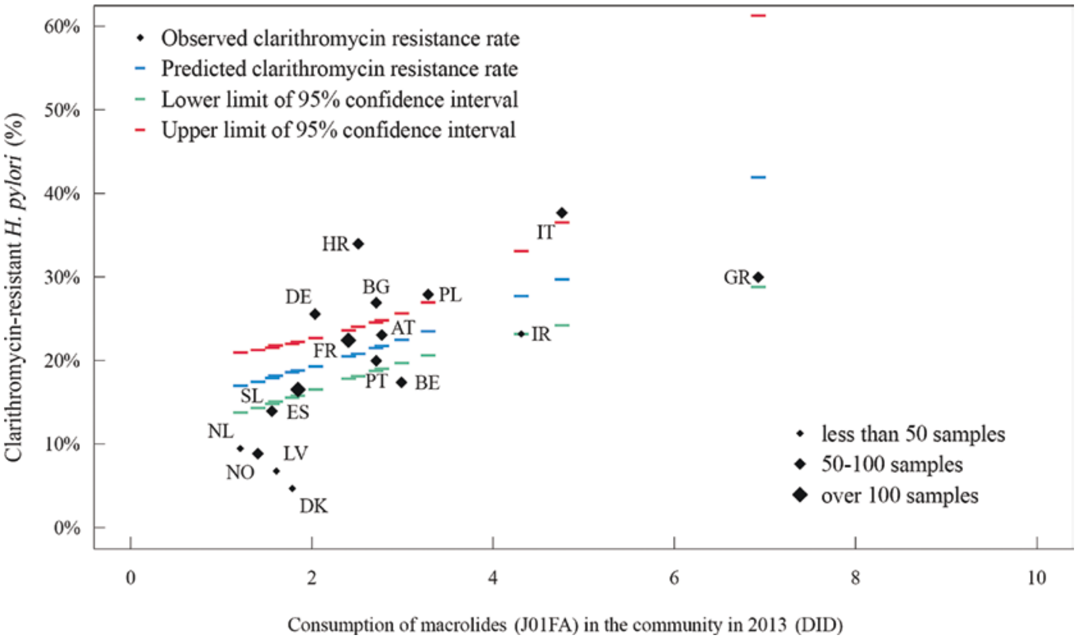

Fig. 38.1 Correlation between macrolide use in 2013 and *H. pylori* resistance to clarithromycin. *AT* Austria; *BE* Belgium; *BG* Bulgaria; *DK* Denmark; *ES* Spain; *FR* France; *DE* Germany; *GR* Greece; *HR* Croatia; *IR* Ireland; *IT* Italy; LV Latvia; *NL* The Netherlands; *NO* Norway; *PL* Poland; *PT* Portugal; *SL* Slovenia

The pool rate of resistance was 31.5% for clarithromycin, 37.6% for levofloxacin, and 42.1% for metronidazole, while it was low as expected for amoxicillin (2.6%), tetracycline (0.87%), and rifabutin (0.17%).

In Latin America, three studies were performed recently, in Honduras [41], Chile [42], and Mexico [43] after the systematic review of 2014 [44]. The level of clarithromycin resistance was still under the usual threshold of 15% in Honduras, while it was over in Chile and Mexico. The trend of clarithromycin resistance showed a significant increase over 20 years in mestizo isolates in Mexico, as well as for levofloxacin. In Honduras, the high levofloxacin and amoxicillin resistances observed reflect a high usage of these antibiotics in the population compared to other nations.

Two studies present data from China, performed in 2017–2021 [45] and 2018–2020 [46] as well as a systematic review and meta-analysis [47]. The results are relatively homogeneous for clarithromycin resistance (34–38.5%) and tetracycline resistance (0–2%). The prevalence of levofloxacin resistance was higher (47.9%) in the study by Shao et al. compared to the others (35%). It was also the case for amoxicillin resistance (9.25%) vs. 2.2% and 3% in the others.

H. pylori antimicrobial resistance was also monitored in 15 centers across Korea in 2017–2018 [48]. They showed the following resistance rates: clarithromycin (17.8%), levofloxacin (37%), tetracycline no resistance, amoxicillin (9.5%), and surprisingly only 29.5% for metronidazole. Another study from a single center which lasted 15 years (2003–2018) [49] showed a significant increase for clarithromycin and metronidazole resistance in contrast to amoxicillin and tetracycline for which the resistance rates were low and stable.

38.6 Conclusions

In summary, the prevalence of *H. pylori* resistance to antibiotics, especially clarithromycin and levofloxacin, is globally increasing in the world, and this is the consequence of the use of antibiotics of these families in the community. Resistance to metronidazole remains high, but this resistance can be overcome. Resistance to amoxicillin and tetracycline, in contrast, is still at a very low level, except probably in rare countries, and gives us the possibility to treat this infection. Given the clinical impact of *H. pylori* resistance especially to clarithromycin, the tools that we have for its detection should lead to an improved *H. pylori* management in the future [50].

References

1. Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet. 1983;1:1273–5.
2. Lind T, Veldhuyzen van Zanten S, Unge P, Spiller R, Bayerdorffer E, O'Morain C, et al. Eradication of *Helicobacter pylori* using one-week triple therapies combining omeprazole with two antimicrobials: the MACH I study. Helicobacter. 1996;1:138–44.
3. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
4. Rice LB. Genetics of resistance. In: Courvalin P, Leclercq R, Rice LB, editors. Antibiogram. Portland: ESKA Publishing/ASM Press; 2010. p. 25–36.
5. Dailidiene D, Bertoli MT, Miciuleviciene J, Mukhopadhyay AK, Ailide G, Pascasio MA, et al. Emergence of tetracycline resistance in *Helicobacter pylori*: multiple mutational changes in 16S ribosomal DNA and other genetic loci. Antimicrob Agents Chemother. 2002;46:3940–6.
6. van Amsterdam K, Bart A, van der Ende A. A *Helicobacter pylori* TolC efflux pump confers resistance to metronidazole. Antimicrob Agents Chemother. 2005;49:1477–82.
7. Versalovic J, Shortridge D, Kibler K, Griffy MV, Beyer J, Flamm RK, et al. Mutations in 23S rRNA are associated with clarithromycin resistance in *Helicobacter pylori*. Antimicrob Agents Chemother. 1996;40:477–80.
8. Occhialini A, Urdaci M, Doucet-Populaire F, Bébéar CM, Lamouliatte H, Mégraud F. Macrolide resistance in *Helicobacter pylori*: rapid detection of point mutations and assays of macrolide binding to ribosomes. Antimicrob Agents Chemother. 1997;41:2724–8.
9. Ménard A, Santos A, Mégraud F, Oleastro M. PCR-restriction fragment length polymorphism can also detect point mutation A2142C in the 23S rRNA gene, associated with *Helicobacter pylori* resistance to clarithromycin. Antimicrob Agents Chemother. 2002;46:1156–7.

10. Moore RA, Beckthold B, Wong S, Kureishi A, Bryan LE. Nucleotide sequence of the *gyr*A gene and characterization of ciprofloxacin-resistant mutants of *Helicobacter pylori*. Antimicrob Agents Chemother. 1995;39:107–11.
11. Tankovic J, Lascols C, Sculo Q, Petit JC, Soussy CJ. Single and double mutations in *gyr*A but not in *gyr*B are associated with low- and high-level fluoroquinolone resistance in *Helicobacter pylori*. Antimicrob Agents Chemother. 2003;47:3942–4.
12. Rhie SY, Park JY, Shin TS, Kim JW, Kim BJ, Kim JG. Discovery of a novel mutation in DNA gyrase and changes in the fluoroquinolone resistance of *Helicobacter pylori* over a 14-year period: a single center study in Korea. Antibiotics (Basel). 2020;9:287.
13. Gerrits MM, Schuijffel D, van Zwet AA, Kuipers EJ, Vandenbroucke-Grauls CM, Kusters JG. Alterations in penicillin-binding protein 1A confer resistance to beta-lactam antibiotics in *Helicobacter pylori*. Antimicrob Agents Chemother. 2002;46:2229–33.
14. Dore MP, Graham DY, Sepulveda AR. Different penicillin-binding protein profiles in amoxicillin-resistant *Helicobacter pylori*. Helicobacter. 1999;4:154–61.
15. Kuo CJ, Ke JN, Kuo T, Lin CY, Hsieh SY, Chiu YF, et al. Multiple amino acid substitutions in penicillin-binding protein-1A confer amoxicillin resistance in refractory *Helicobacter pylori* infection. J Microbiol Immunol Infect. 2022;56:40–7.
16. Tran TT, Nguyen AT, Quach DT, Pham DT, Cao NM, Nguyen UT, et al. Emergence of amoxicillin resistance and identification of novel mutations of the *pbp1A* gene in *Helicobacter pylori* in Vietnam. BMC Microbiol. 2022;22:41.
17. Gerrits MM, de Zoete MR, Arents NL, Kuipers EJ, Kusters JG. 16S rRNA mutation-mediated tetracycline resistance in *Helicobacter pylori*. Antimicrob Agents Chemother. 2002;46:2996–3000.
18. Trieber CA, Taylor DE. Mutations in the 16S rRNA genes of *Helicobacter pylori* mediate resistance to tetracycline. J Bacteriol. 2002;184:2131–40.
19. Heep M, Beck D, Bayerdörffer E, Lehn N. Rifampin and rifabutin resistance mechanism in *Helicobacter pylori*. Antimicrob Agents Chemother. 1999;43:1497–9.
20. Hoffman PS, Goodwin A, Johnsen J, Magee K, Veldhuyzen van Zanten SJ. Metabolic activities of metronidazole-sensitive and -resistant strains of *Helicobacter pylori*: repression of pyruvate oxidoreductase and expression of isocitrate lyase activity correlate with resistance. J Bacteriol. 1996;178:4822–9.
21. Marais A, Bilardi C, Cantet F, Mendz GL, Mégraud F. Characterization of the genes *rdx*A and *frx*A involved in metronidazole resistance in *Helicobacter pylori*. Res Microbiol. 2003;154:137–44.
22. Glupczynski Y, Broutet N, Cantagrel A, Andersen LP, Alarcon T, López-Brea M, et al. Comparison of the E test and agar dilution method for antimicrobial susceptibility testing of *Helicobacter pylori*. Eur J Clin Microbiol Infect Dis. 2002;21:549–52.
23. Attaran B, Falsafi T, Ghorbanmehr N. Effect of biofilm formation by clinical isolates of *Helicobacter pylori* on the efflux-mediated resistance to commonly used antibiotics. World J Gastroenterol. 2017;23:1163–70.
24. Piccolomini R, Di Bonaventura G, Catamo G, Carbone F, Neri M. Comparative evaluation of the E test, agar dilution, and broth microdilution for testing susceptibilities of *Helicobacter pylori* strains to 20 antimicrobial agents. J Clin Microbiol. 1997;35:1842–6.
25. Coudron PE, Stratton CW. Factors affecting growth and susceptibility testing of *Helicobacter pylori* in liquid media. J Clin Microbiol. 1995;33:1028–30.
26. Tang X, Shen Y, Hu R, Yang T, Benghezal M, Li H, et al. Re-assessment of the disk diffusion technique for routine antimicrobial susceptibility testing for *Helicobacter pylori*. Helicobacter. 2020;25:e12703.
27. Mégraud F, Bénéjat L, Ontsira Ngoyi EN, Lehours P. Molecular approaches to identify *Helicobacter pylori* antimicrobial resistance. Gastroenterol Clin N Am. 2015;44:577–96.
28. Oleastro M, Ménard A, Santos A, Lamouliatte H, Monteiro L, Barthélémy P, et al. Real-time PCR assay for rapid and accurate detection of point mutations conferring resistance to clarithromycin in *Helicobacter pylori*. J Clin Microbiol. 2003;41:397–402.
29. Bénéjat L, Ducournau A, Domingues-Martins C, Lecoeur M, Blosse A, Mégraud F, et al. Adaptation of an in-house PCR for the detection of *Helicobacter pylori* and the mutations associated with macrolide resistance into ready-to-use PCR microwell strips. Helicobacter. 2021;26:e12855.
30. Bénéjat L, Giese A, Lescaudron Z, Bonnac J, Ducournau A, Bessède E, et al. Automation of RIDA®GENE *Helicobacter pylori* PCR on the BD MAX™ system. Eur J Clin Microbiol Infect Dis. 2022;41:875–9.
31. Tsuda M, Watanabe Y, Oikawa R, Watanabe R, Higashino M, Kubo K, et al. Clinical evaluation of a novel molecular diagnosis kit for detecting *Helicobacter pylori* and clarithromycin-resistant using intragastric fluid. Helicobacter. 2022;27:e12933.
32. Schabereiter-Gurtner C, Hirschl AM, Dragosics B, Hufnagl P, Puz S, Kovách Z, et al. Novel real-time PCR assay for detection of *Helicobacter pylori* infection and simultaneous clarithromycin susceptibility testing of stool and biopsy specimens. J Clin Microbiol. 2004;42:4512–8.
33. Monteiro L, Gras N, Vidal R, Cabrita J, Megraud F. Detection of *Helicobacter pylori* DNA in human feces by PCR: DNA stability and removal of inhibitors. J Microbiol Methods. 2001;45:89–94.
34. Glocker E, Kist M. Rapid detection of point mutations in the *gyr*A gene of *Helicobacter pylori* conferring resistance to ciprofloxacin by a fluorescence resonance energy transfer-based real-time PCR approach. J Clin Microbiol. 2004;42:2241–6.
35. Hulten KG, Genta RM, Kalfus IN, Zhou Y, Zhang H, Graham DY. Comparison of culture with antibiogram to next-generation sequencing using bacterial isolates

and formalin-fixed, paraffin-embedded gastric biopsies. Gastroenterology. 2021;161:1433–42.

36. Moss SF, Dang LP, Chua D, Sobrado J, Zhou Y, Graham DY. Comparable results of *Helicobacter pylori* antibiotic resistance testing of stools vs gastric biopsies using next-generation sequencing. Gastroenterology. 2022;162:2095–7.
37. Megraud FH. *Pylori* antibiotic resistance: prevalence, importance, and advances in testing. Gut. 2004;53:1374–84.
38. Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, et al. *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut. 2013;62:34–42.
39. Megraud F, Bruyndonckx R, Coenen S, Wittkop L, Huang TD, Hoebeke M, et al. *Helicobacter pylori* resistance to antibiotics in Europe in 2018 and its relationship to antibiotic consumption in the community. Gut. 2021;70:1815–22.
40. Ho JJC, Navarro M, Sawyer K, Elfanagely Y, Moss SF. *Helicobacter pylori* antibiotic resistance in the United States between 2011 and 2021: a systematic review and meta-analysis. Am J Gastroenterol. 2022;117:1221–30.
41. Ortiz V, Estevez-Ordonez D, Montalvan-Sanchez E, Urrutia-Argueta S, Israel D, Krishna US, et al. *Helicobacter pylori* antimicrobial resistance and antibiotic consumption in the low-resource Central America setting. Helicobacter. 2019;24:e12595.
42. Arenas A, Serrano C, Quiñones L, Harris P, Sandoval M, Lavanderos M, et al. High prevalence of clarithromycin resistance and effect on *Helicobacter pylori* eradication in a population from Santiago, Chile: cohort study and meta-analysis. Sci Rep. 2019;9:20070.
43. Camorlinga-Ponce M, Gómez-Delgado A, Aguilar-Zamora E, Torres RC, Giono-Cerezo S, Escobar-Ogaz A, et al. Phenotypic and genotypic antibiotic resistance patterns in *Helicobacter pylori* strains from ethnically diverse population in México. Front Cell Infect Microbiol. 2021;10:539115.
44. Camargo MC, Garcia A, Riquelme A, Otero W, Camargo CA, Hernandez-Garcia T, et al. The problem of *Helicobacter pylori* resistance to antibiotics: a systematic review in Latin America. Am J Gastroenterol. 2014;109:485–95.
45. Shao Y, Lin Y, Wang B, Miao M, Ye G. Antibiotic resistance status of *Helicobacter pylori* strains isolated from initial eradication patients in Ningbo, China, from 2017 to 2021. Helicobacter. 2022;27:e12920.
46. Zhong Z, Zhang Z, Wang J, Hu Y, Mi Y, He B, et al. A retrospective study of the antibiotic-resistant phenotypes and genotypes of *Helicobacter pylori* strains in China. Am J Cancer Res. 2021;11:5027–37.
47. Chen J, Li P, Huang Y, Guo Y, Ding Z, Lu H. Primary antibiotic resistance of *Helicobacter pylori* in different regions of China: a systematic review and meta-analysis. Pathogens. 2022;11:786.
48. Lee JH, Ahn JY, Choi KD, Jung HY, Kim JM, Baik GH, et al. Nationwide antibiotic resistance mapping of *Helicobacter pylori* in Korea: a prospective multicenter study. Helicobacter. 2019;24:e12592.
49. Lee JY, Kim N, Nam RH, In Choi S, Lee JW, Lee DH. Primary and secondary antibiotic resistance of *Helicobacter pylori* in Korea from 2003 to 2018. Helicobacter. 2019;24:e12660.
50. Argueta EA, Ho JJC, Elfanagely Y, D'Agata E, Moss SF. Clinical implication of drug resistance for *H. pylori* management. Antibiotics (Basel). 2022;11:1684.

Clarithromycin

39

Jung Won Lee

Abstract

Clarithromycin is a macrolide antimicrobial agent that is the main component of proton pump inhibitor (PPI) triple *Helicobacter pylori* (*H. pylori*) eradication therapy. Moreover, clarithromycin resistance is the most critical component to determine either the success or the failure of the eradication. Recently, clarithromycin resistance has rapidly increased. Clarithromycin resistance mechanisms mostly depend on 23S rRNA gene mutations, and A2143G point mutation is the most critical. Thus, the pretreatment determination of clarithromycin susceptibility can guarantee eradication success. Moreover, genetic epidemiologic investigations on resistance-inducing mutations contributed to the development of molecular diagnostic tests for rapid decision for clarithromycin susceptibility. Meanwhile, further research is needed to identify the unknown mechanism of clarithromycin resistance.

Keywords

Helicobacter pylori · Antibiotic resistance
Clarithromycin

39.1 Introduction

The combination of proton pump inhibitor (PPI), amoxicillin, and clarithromycin for 7 days has been globally established as the first-line eradication therapy of *Helicobacter pylori* (*H. pylori*) [1, 2]. However, this combination therapy could no longer show the same efficacy as the past due to clarithromycin resistance. Therefore, some countries no longer adopt clarithromycin-containing triple therapy as the first-line eradication therapy. On the other hand, countries like the Republic of Korea still maintain clarithromycin-containing triple therapy as the first-line treatment due to the difficulty of establishing an alternative therapy caused by high antimicrobial resistance on multiple antimicrobial agents. Like this, clarithromycin has been the core of most concise and effective *H. pylori* treatment. The unexpected rapid increase in clarithromycin resistance is a great challenge in establishing a new eradication strategy.

Clarithromycin is a macrolide antimicrobial agent that includes a large tetradecagonal ring inside its molecular structure. Macrolide antimicrobial agents in the same class include erythromycin, clarithromycin, and roxithromycin. Clarithromycin is different from erythromycin because the 6-hydroxyl group of clarithromycin is methylated, unlike that of erythromycin (Fig. 39.1). Because of this methylation, clarithromycin is more stable under acidic condition and has better tissue penetration efficacy than

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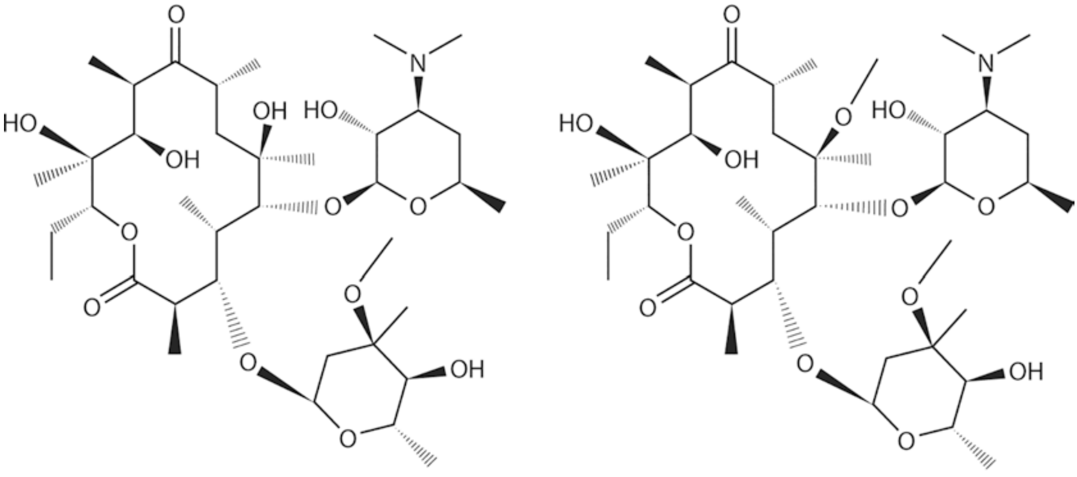

Erythromycin

Clarithromycin

Fig. 39.1 Molecular structures of erythromycin and clarithromycin

erythromycin and has been core for first-line *H. pylori* eradication treatment. Clarithromycin was introduced in Korea in 1997, and it has been the fundamental antibiotic for *H. pylori* treatment [3].

In this chapter, the present author prepared the epidemiology and mechanism of clarithromycin resistance. Moreover, we can overview the current status of the studies regarding clarithromycin resistance and future perspectives.

39.2 The Antimicrobial Mechanism of Clarithromycin

Clarithromycin could be classified into a protein synthesis inhibitor of bacteria. Clarithromycin mainly shows a bacteriostatic characteristic, which means the inhibition of bacterial growth, instead of bactericidal activity. The main mechanism of clarithromycin is to attach to domain V peptidyl transferase ring of ribosomal RNA (rRNA) structure and then inhibits the protein synthesis of bacteria. This inhibition prevents amino acid chain structure formations, and all macrolide antibiotics commonly have this mechanism. In addition, clarithromycin tends to be better absorbed to stomach mucosa and shows better stability in acidic condition [4].

39.3 The Prevalence and Trends of Clarithromycin Resistance

Clarithromycin has been the core of *H. pylori* eradication therapy [5]. After the eradication treatment for *H. pylori* was introduced, clarithromycin-containing therapy has been the most convenient and effective treatment. However, worldwide empirical use of clarithromycin-containing therapy is believed to be associated with worldwide development of clarithromycin resistance. Clarithromycin resistance is considered to be the biggest problem of eradication therapy in the United States, Europe, and Asian countries.

A study on worldwide prevalence of clarithromycin resistance was published in 2018 sponsored by the World Health Organization (WHO). The study included 66,142 isolates from a total of 178 studies conducted in 65 countries. The clarithromycin resistance in the meta-analysis in this study exceeded 15% in all regions except for the U.S. and South-East Asia regions. Moreover, it was also accompanied by an increasing trend in resistance in most regions [6] (Fig. 39.2).

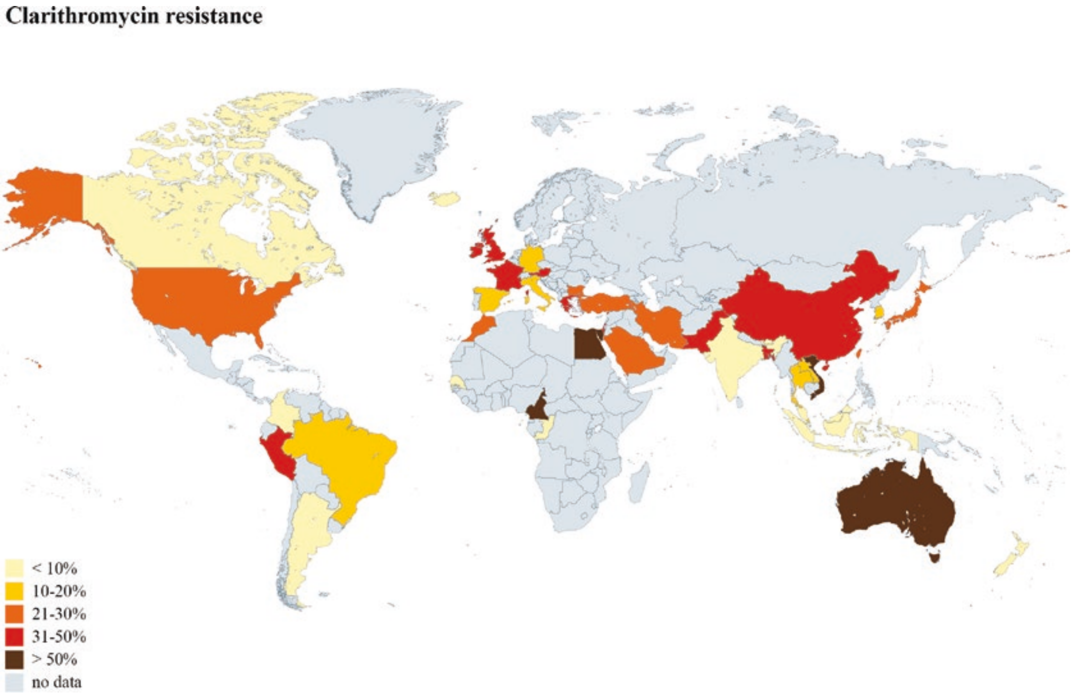

Fig. 39.2 Pooled prevalence (2006–2016) of resistance to clarithromycin. (Adapted from Savoldi et al. [6])

United States showed around 10.0% of prevalence during 1990s, and the rate has increased to 15% at 2010s. At 2023, the results of a study conducted on 907 subjects recruited from 108 institutions across the United States were published and the overall clarithromycin resistance in the United States was reported as 22.2% [7]. For this result, the empirical use of clarithromycin-containing therapy in the United States was expected to be unacceptable. Meanwhile, the prevalence rates were significantly different among European nations (Fig. 39.3); Italy (36.9%), Croatia (34.6%), and Greece (30.0%) showed very high rates of resistance compared to the Netherlands (9.2%), Denmark (4.8%), and Norway (8.9%) [8, 9]. These differences are caused by different antibiotic prescription patterns among the nations, and nations with especially high macrolide antibiotic uses on respiratory diseases show higher resistances [9].

Clarithromycin resistance was also high in Asia. In a systematic review published in 2017, most of the major Asian countries, including China (26%) and Japan (19%), showed results exceeding 15% [10].

Clarithromycin was introduced in Korea in 1997. The first systematic report on clarithromycin resistance in Korea was introduced by Kim et al. in 2005. The clarithromycin resistance on *H. pylori* in Korea was not reported in 1987, and low incidence (2.8%) of clarithromycin resistance was reported in 1994. However, the resistance was increased by 13.5% in 2003. In addition, according to the study on the changes of clarithromycin resistance prevalence of 347 patients from 2003 to 2012 in Gyeonggi-do, Korea, clarithromycin resistance rates were 22.9% from 2003 to 2005, 25.5% from 2006 to 2008, and 37.0% from 2009 to 2012, which show rapid increase [11]. In the most recent resistance trend report by 2022, there was a continuous increase (primary resistance reached 31%). However, the statistical significance was weakened, suggesting that resistance in Korea seems to be close on a plateau [12] (Fig. 39.4). Although these rates were obtained from tertiary-referral hospitals, these increased primary and secondary prevalence rates clearly indicate

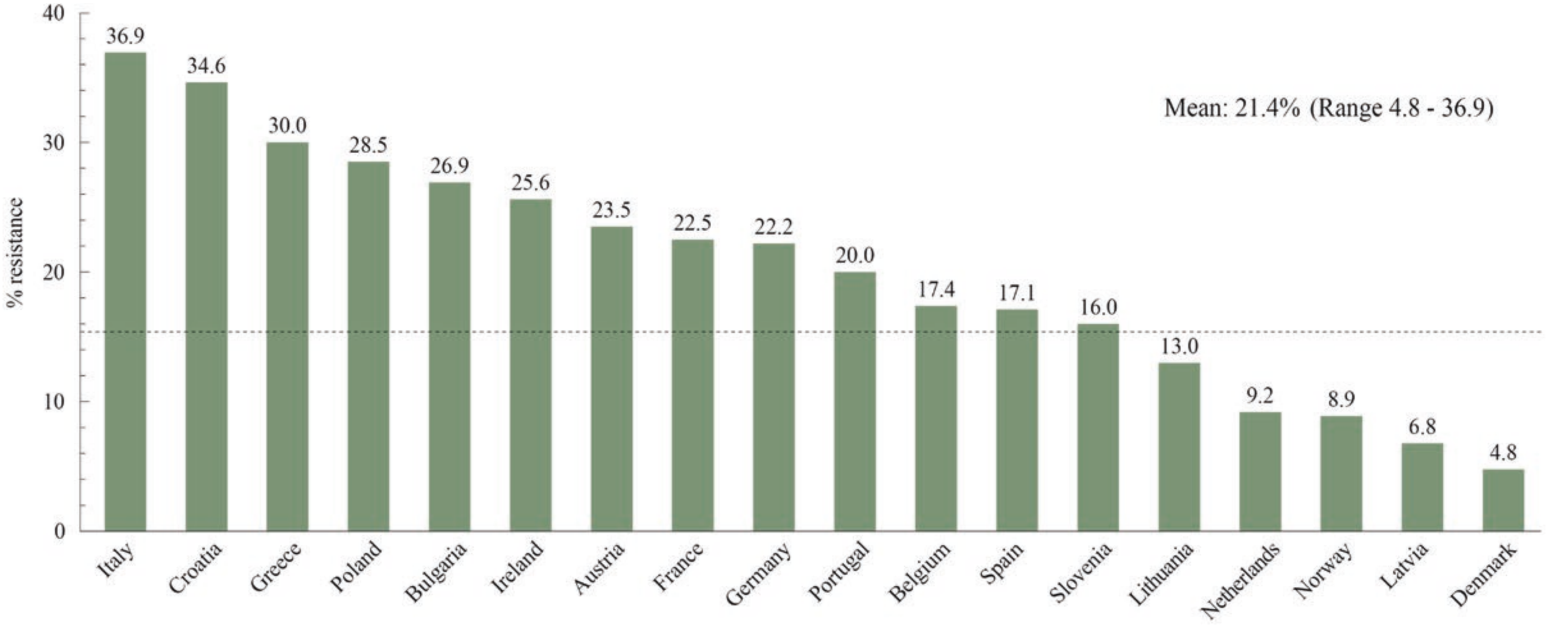

Fig. 39.3 The current clarithromycin resistance rates in Europe. (Adapted from Megraud et al. [8])

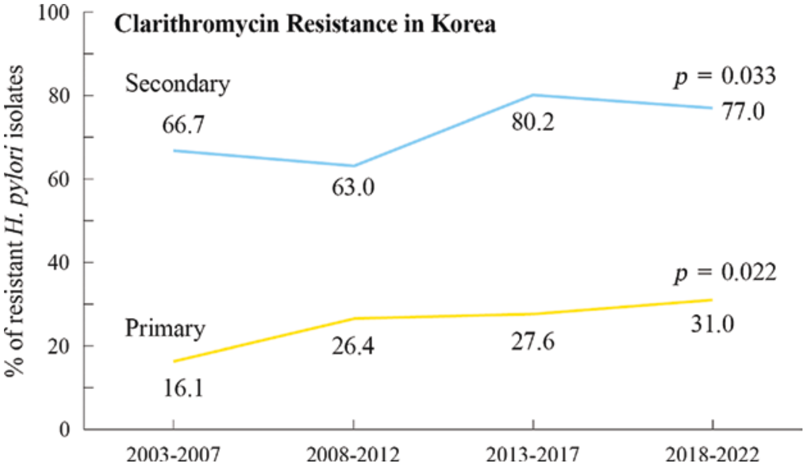

Fig. 39.4 The prevalence and trends of primary and secondary clarithromycin resistance rates in Korea. (Adapted from Lee et al. [11, 12])

the increased macrolide resistance. In addition, this study identified that the previous medical history of antibiotic treatment is a meaningful risk factor of antibiotic resistance, which indicates that the exposure to antibiotics is related to the development of resistance. Nevertheless, macrolide monotherapy on respiratory disorders is identified to be no longer effective, giving consistent interests that the change of clarithromycin resistance rate is necessary, since macrolide antibiotics are still widely used in community settings. Meanwhile, clarithromycin resistances of three different regions were from 12.5 to 40.0% based on the multicenter study in Korea by Kim et al. in 2011 [13] and Lee et al. in 2019 [9, 14], so this result suggests that the identification of resistance rates among local communities should be done prior to the clarithromycin-containing eradication treatment.

39.4 Diagnosing Clarithromycin Resistance

39.4.1 Diagnosing Clarithromycin Resistance: Conventional Culture and Susceptibility

The method of diagnosing clarithromycin resistance is relatively well established. The most popular tests that are used by current researchers are agar-dilution test and E-test. The criteria for the antibiotic resistance of bacteria follow the criteria of internationally certified antibiotic resistance test standards, which are the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) in the United States and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in Europe. CLSI recommends agar-dilution test as a gold standard for clarithromycin resistance, and the test determines a resistant-positive result if minimal inhibitory concentration (MIC) is >1 μg/mL. However, many researchers prefer E-test due to financial and time issues, and they often set their standards of determining resistance with minimum MIC values higher than 1 μg/mL. E-test is cheaper and easier to perform compared to agar-dilution test, but the MIC determination is less accurate and CLSI does not recommend this test. This is a problem that needs consensus among researchers based on precise evidences.

The most critical disadvantage of the culture and susceptibility test for diagnosing resistance is that the culture success rate is only 70–80% [15]. Because of this, routine use is expected to be difficult in the future, and it is considered that it will be used only for patients with eradication failure for several times.

39.4.2 Diagnosing Clarithromycin Resistance: Molecular Tests

Because of low yield of conventional cultures and susceptibility, various molecular tests have been invented for rapid resistance determination of clarithromycin resistance. Exceptionally, clarithromycin resistance is mainly caused by target alteration in antibiotic attach sites at bacterial ribosome [16, 17]. In particular, the presence of a small number of point mutations in 23SrRNA is highly correlated with phenotypic resistance. This motivated the development of molecular diagnostic methods. The PCR method using gastric biopsy specimens is currently producing the most reliable and confident results. In a recent systematic review, the A2142C, A2142G, or A2143G mutation for the determination of clarithromycin resistance was found to have both sensitivity and specificity of 96% [18]. Recently, it has been reported that the PCR method is useful for samples of a rapid urease test kit [19]. To further reduce invasiveness, PCR methods using stool samples are being investigated [20]. Moreover, next-generation sequencing (NGS) shows promising results [21].

39.5 Mechanisms of Clarithromycin Resistance

Clarithromycin resistance of *H. pylori* is mainly about the target alteration for antibiotics, like other Gram-negative bacteria. The target alteration occurs in 23S ribosome, which is the component of 50S ribosome, and the 23S ribosome is crucial for *H. pylori* protein synthesis. This alteration is caused by either a posttranscriptional methylation or the alteration of 23S rRNA sequences. Also, the activation of efflux pump is an important mechanism [22].

Clarithromycin target alteration takes the most important role for clarithromycin resistance, among all of the known macrolide antibiotic resistance mechanisms. Especially, the alterations of several 23S rRNA sequences induce the resistance of *H. pylori*. This alteration reduces the adhesion of macrolide antibiotics on their targets, so the resistance occurs. Studies about the target alteration mechanism have been reported since mid-1990s. In 1996, frequent transformations that the replacements of nuclic acid, adenine, of 2142 and 2143 among 23S rRNA sequences into cytosine or guanine were reported at first. Particularly, A2142C, A2142G, or A2143G mutation is identified to be highly related with high resistance on macrolide antibiotics [23] (Fig. 39.5). Also, T2182C mutations in macrolide-resistant *H. pylori* were frequently reported. Other mutations, such as A2136G, G2141A, and A2144T, were also reported, but these are considered to be less influential, since they are not consistently reported by later studies.

Another important resistance mechanism is the activation of efflux pump. There are five well-known efflux pump systems so far, which are multidrug and toxic compound extrusion proteins, major facilitator superfamily, ATP-binding cassette superfamily, and resistance-nodulation cell division (RND) [24–26]. Especially, *H. pylori* is known to have RND-type multidrug efflux pump. In 2005, *hp0605-hp0607*, *hp0971-hp0969*, *hp1327-hp1329*, and *hp1489-hp1487* are reported to be the candidates of RND-type multidrug efflux pump resistance of *H. pylori*. Also, *hp0605-hp0607* (*hefA*) overexpression and its direct interference on the expression of *H. pylori* multidrug resistance (MDR) efflux pump have been identified. The *hefA* gene-expressed MDR efflux pump is initially known to exist in bacteria that show multidrug resistance due to external stimulations, like repetitive exposure to antibiotics. However, there is no study in Korea regarding this issue so far, and the same studies

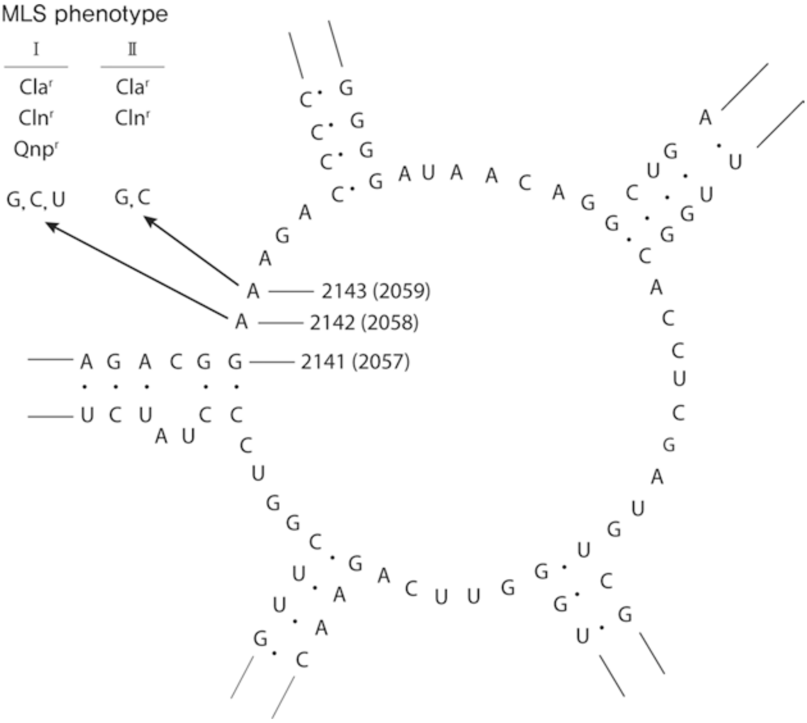

Fig. 39.5 Domain V (peptidyltransferase loop) structure of 23S rRNA gene. (Adapted from Wang and Taylor [23])

for explaining the resistance mechanism are only limited to laboratory levels outside Korea.

39.6 Current Clarithromycin Resistance Studies and Its Prospection

39.6.1 Current Clarithromycin Resistance Studies

Kim et al. confirmed 23S rRNA resistance gene mutations among clarithromycin-resistant *H. pylori* colonies of Korean patients from 2003 to 2006 via DNA sequencing [27]. As a result, 71.4% of patients who previously received *H. pylori* eradication therapy showed A2143G transformation, and the rest 28.6% of patients showed T2182C transformation. Also, both A2143G and T2182C mutations coexisted in 70.3% of secondary colonies with previous *H. pylori* antibacterial treatments. In conclusion, it is confirmed that a past antibiotic exposure contributes to high resistance as well as complicated mutation, and the most important genetic mutation of resistance is identified to be A2143G (Table 39.1).

Table 39.1 The current distributions of 23S rRNA resistance genes in Korea

Genotypes of 23S rRNA mutation	No. of isolates (%)	
	Primary isolates	Secondary isolates
A2143G	15 (71.4)	5 (7.8)
T2182C	6 (28.6)	5 (7.8)
A2143G + T2182C		45 (70.3)
A2143G + T2182C + T2190C		4 (6.3)
A2143G + T2182C + C2195T		3 (4.7)
A2143G + T2182C + A2223G		2 (3.1)
Total	21 (100)	64 (100)

Adapted from Kim et al.

Adapted from Kim et al. [27]

Along with those resistance mechanisms, Hwang et al. reported about the role of A2143G genetic mutation in 2010 [17]. This study directly analyzed how point mutation contributes to resistance expression and the success of antibacterial treatment [17]. Among six mutated A2143G *H. pylori* colonies in the study, none of those was susceptible to clarithromycin-included standard triple therapy. Thus, it could be concluded that A2143G transformation is the important transformation to determine the success of eradication therapy. In 2013, there was a report on the relationship between eradication results and 23S rRNA resistance mutated genes, based on 110 patients who had *H. pylori* eradication therapy in Jeju Island, Korea. Among those 110 patients, only 23 patients (18.1%) had A2143G mutation. However, 87.5% of unsuccessful antibacterial therapy results could be seen among patients with A2143G transformed colonies, which confirms that A2143G mutation status significantly affects the successful eradication therapy.

As mentioned in previous sections, clarithromycin resistance is the worldwide and nationwide problem [6]. Several investigators keep trying new antimicrobial agents or regimens, such as the sequential or concomitant treatment. Recently, several alternative therapies demonstrate good results and are officially recommended by several guidelines as first-line treatment [2, 3]. However, clarithromycin itself can be used as a very effective antibiotic, as long as there is no resistance [28]. Several investigators agreed that the clarithromycin-containing triple therapy can be still used as an effective treatment with minimal adverse effects, if there is no clarithromycin resistance [2, 28]. Thus, many investigators have focused on developing new tests to easily determine clarithromycin resistance [20].

Molecular diagnostic methods about the genes responsible for resistance have been studied since the late 1990s. Most of the diagnoses were the modifications of polymerase chain reaction (PCR) [29]. However, these modifications did not show any clinically superiority to previous MIC tests in terms of time and financial conditions. This is because patients who failed their first eradication therapies usually get successful eradications after the second or third treatment, and performing antibiotic susceptibility tests after treatment failure was not easily done all the time.

GenoType HelicoDR® test was a remarkable diagnostic test published in 2009 because it is a mixture of PCR and DNA strip method, and the test could diagnose A2142C and A2143G transformations from a stomach mucosal biopsy tissue sample within 6 h [30]. Also, this test can diagnose *gyrA* gene mutation, which is a quinolone resistance gene, so the test was considered to be effective. A GenoType HelicoDR® test study was conducted to identify its clinical efficacy, and the test predicted resistance gene expression with meaningful selectivity and specificity. This study showed that the test can be used to predict an eradication failure due to clarithromycin resistance, but there are still numerous problems with false-positive results [16]. Another promising test would be dual-priming oligonucleotide (DPO)-based multiplex PCR of Seegene Inc. that was reported by Megraud et al. of France [31]. Megraud et al. utilized 127 gastric mucosal tissues to compare the difference with E-test in this report, and there was 95.3% conformity between the two tests, which can be accepted as effective. However, further studies on DPO-based multiplex PCR are not actively reported nowadays. Recently, a simplified molecular test has been launched to confirm the presence of *H. pylori* clarithromycin-resistant mutant genes via DNA sequencing by a company in Korea. This test takes approximately 3 days, but it uses DNA sequencing that allows various mutant identifications regardless of their types; currently, PCR test is covered by national health care insurance of Korea and now available at worldwide [3, 32].

The achievements of this DPO-based PCR method are remarkable. Due to the increased PCR test capacity by the COVID-19 pandemic in 2020–2022, PCR testing for clarithromycin resistance has become more easier and common [2]. Many guidelines recommend performing a susceptibility test using molecular methods before eradication, especially in areas with high prevalence of clarithromycin resistance [2, 3]. These pretreatment susceptibility tests also appear to be effective in a cost-effective analysis [33].

39.6.2 The Prospect About Future Clarithromycin Resistance Studies

As mentioned in the previous section, the current worldwide prevalence of clarithromycin resistance is so high that the use of empirical clarithromycin-containing therapy is not recommended in most countries [2, 3, 7, 8]. However, bismuth-quadruple therapy, which is considered an alternative first-line therapy, also has a unacceptable success rate in patients with multiresistance to clarithromycin and metronidazole as first-line eradication therapy. Therefore, investigators need to find an answer to how to set the eradication strategy in the case of patients with multiple antimicrobial resistance.

Recently, resistance detection method using next-genome sequencing has been studied [34]. In particular, it is very useful for detecting point mutations critical to resistance development, and it is well known that decisive mutations can be detected for several antimicrobial agents simultaneously. Moreover, it has recently been reported that next-genome sequencing by stool specimens demonstrates similar efficacy compared to gastric biopsy specimens [20, 21]. Meanwhile, most of researchers have mainly focused on the antibiotic target alteration mechanism, while they have given less interest on other mechanisms. Thus, studies on new resistance mechanisms are performed by using next-genome sequencing methods. In 2014, Yoshino Yamaoka research team used next-genome sequencing to analyze low-level resistance-related genes, except for A2143G mutation that is related to high-level resistance. This study reported the existence of new resistance gene by confirming that *hp1048* (*infB*) and *hp1314* (*rp122*) sequence mutations induce resistance on ATCC 26695 *H. pylori* colonies via transformation experiment [35]. Particularly, a very high resistance, which is represented as MIC >256 μg/mL, could be seen, if resistant on both *infB* and *rp122* genes.

In 2014, Toshihito Tanahashi research team reported a new genetic mutation other than efflux pump-related mutation, by using whole-genome sequencing. Especially among RND-type efflux family genes, single-nucleotide variants of *hp0605-hp0607*, *hp0971-hp0969*, *hp1327-hp1329*, and *hp1489-hp487* were identified and their sequences were reported [36]. Along with these discoveries, a turning point to overcome resistance based on efflux pump mechanism is expected. Also, there is a report that a biofilm formation is significantly related to a resistance expression, so new research results should be focused as well [37].

In the cases of quinolone, new medications are continuously developed, and most of these quinolones insist they could overcome resistance gene mutations [38–40]. In comparison, it is unfortunate that macrolide antibiotics still could not demonstrate new medication developments. It is necessary to develop new macrolide antibiotics useful against *H. pylori*. Above all, improved stability in an acidic environment is most important.

In the latest European guidelines, Maastricht VI/Florence guideline still maintains the maximum limitation of local community prevalence that clarithromycin can be used for first-line eradication therapy, as 15% [2]. In other words, the study advised to minimize clarithromycin use during first-line eradication therapy, if the prevalence of a community setting is higher than 15%. However, this recommendation is difficult to be accepted in Korea because of high resistance rate of multiple antimicrobial agents [12]. Especially, clarithromycin can be still effectively used for eradication treatments for susceptible cases even for 7-days duration [28]. In addition, questions continue to be raised as to whether the 15% is appropriate [41].

39.7 Conclusions

Clarithromycin is the core of *H. pylori* eradication therapy due to its minimal adverse effects and outstanding antimicrobial efficacy, by taking two oral tablets per day. However, there are many limitations on using clarithromycin by the recent increasing trends of resistance. The clarithromycin-resistant mechanisms mostly depend on 23S rRNA gene mutations. The most significant gene mutations are A2142C, A2142G, or A2143G mutation, and this mutation is closely involved in the failure of clarithromycin-containing therapy. Because of high prevalence of clarithromycin-resistance, pretreatment susceptibility test is necessary in most countries. Currently, molecular tests using PCR have become very useful and promising. Epidemiological studies for mutations which were responsible for antimicrobial resistance should be continued because these studies could provide effective information which could be useful for the development of novel molecular diagnostic tool or antimicrobial agent.

References

1. Graham DY, Fischbach L. *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. Gut. 2010;59:1143–53.
2. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
3. Jung HK, Kang SJ, Lee YC, Yang HJ, Park SY, Shin CM, et al. Evidence-based guidelines for the treatment of *Helicobacter pylori* infection in Korea 2020. Gut Liver. 2021;15:168–95.
4. Versalovic J, Osato MS, Spakovsky K, Dore MP, Reddy R, Stone GG, et al. Point mutations in the 23S rRNA gene of *Helicobacter pylori* associated with different levels of clarithromycin resistance. J Antimicrob Chemother. 1997;40:283–6.
5. Vakil NH. *Pylori* treatment: new wine in old bottles? Am J Gastroenterol. 2009;104:26–30.
6. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of antibiotic resistance in *Helicobacter pylori*: a systematic review and meta-analysis in World Health Organization regions. Gastroenterology. 2018;155:1372–82.
7. Megraud F, Graham DY, Howden CW, Trevino E, Weissfeld A, Hunt B, et al. Rates of antimicrobial resistance in *Helicobacter pylori* isolates from clinical trial patients across the US and Europe. Am J Gastroenterol. 2023;118:269–75.
8. Megraud F, Bruyndonckx R, Coenen S, Wittkop L, Huang TD, Hoebeke M, et al. *Helicobacter pylori* resistance to antibiotics in Europe in 2018 and its relationship to antibiotic consumption in the community. Gut. 2021;70:1815–22.
9. Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, et al. *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut. 2013;62:34–42.
10. Kuo YT, Liou JM, El-Omar EM, Wu JY, Leow AHR, Goh KL, et al. Primary antibiotic resistance in *Helicobacter pylori* in the Asia-Pacific region: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2017;2:707–15.
11. Lee JW, Kim N, Kim JM, Nam RH, Chang H, Kim JY, et al. Prevalence of primary and secondary antimicrobial resistance of *Helicobacter pylori* in Korea from 2003 through 2012. Helicobacter. 2013;18:206–14.
12. Lee JW, Kim N, Choi SI, Jang JY, Song CH, Nam RH, et al. Prevalence and trends of multiple antimicrobial resistance of *Helicobacter pylori* in one tertiary hospital for 20 years in Korea. Helicobacter. 2023;28:e12939.
13. Kim JY, Kim NY, Kim SJ, Baik GH, Kim GH, Kim JM, et al. Regional difference of antibiotic resistance of *Helicobacter pylori* strains in Korea. Korean J Gastroenterol. 2011;57:221–9.
14. Lee JH, Ahn JY, Choi KD, Jung HY, Kim JM, Baik GH, et al. Nationwide antibiotic resistance mapping of *Helicobacter pylori* in Korea: a prospective multicenter study. Helicobacter. 2019;24:e12592.
15. Gisbert JP. Empirical or susceptibility-guided treatment for *Helicobacter pylori* infection? A comprehensive review. Therap Adv Gastroenterol. 2020;13:1756284820968736.
16. Lee JW, Kim N, Nam RH, Park JH, Choi YJ, Kim JM, et al. GenoType HelicoDR test in the determination of antimicrobial resistance of *Helicobacter pylori* in Korea. Scand J Gastroenterol. 2014;49:1058–67.
17. Hwang TJ, Kim N, Kim HB, Lee BH, Nam RH, Park JH, et al. Change in antibiotic resistance of *Helicobacter pylori* strains and the effect of A2143G point mutation of 23S rRNA on the eradication of *H. pylori* in a single center of Korea. J Clin Gastroenterol. 2010;44:536–43.
18. Wang YH, Li Z, Wang L, Zhu-Ge LY, Zhao RL, Wu S, et al. A systematic review and meta-analysis of genotypic methods for detecting antibiotic resistance in *Helicobacter pylori*. Helicobacter. 2018;23:e12467.
19. Li Y, Rimbara E, Thirumurthi S, Trespalacios A, Reddy R, Sabounchi S, et al. Detection of clarithromycin resistance in *Helicobacter pylori* following noncryogenic storage of rapid urease tests for 30 days. J Dig Dis. 2012;13:54–9.
20. Moss SF, Dang LP, Chua D, Sobrado J, Zhou Y, Graham DY. Comparable results of *Helicobacter pylori* antibiotic resistance testing of stools vs gastric biopsies using next-generation sequencing. Gastroenterology. 2022;162:2095–7.
21. Hulten KG, Genta RM, Kalfus IN, Zhou Y, Zhang H, Graham DY. Comparison of culture with antibiogram to next-generation sequencing using bacterial isolates and formalin-fixed, paraffin-embedded gastric biopsies. Gastroenterology. 2021;161:1433–42.
22. Gerrits MM, van Vliet AH, Kuipers EJ, Kusters JG. *Helicobacter pylori* and antimicrobial resistance: molecular mechanisms and clinical implications. Lancet Infect Dis. 2006;6:699–709.

23. Wang G, Taylor DE. Site-specific mutations in the 23S rRNA gene of *Helicobacter pylori* confer two types of resistance to macrolide-lincosamide-streptogramin B antibiotics. Antimicrob Agents Chemother. 1998;42:1952–8.
24. Kutschke A, de Jonge BL. Compound efflux in *Helicobacter pylori*. Antimicrob Agents Chemother. 2005;49:3009–10.
25. Liu ZQ, Zheng PY, Yang PC. Efflux pump gene hefA of *Helicobacter pylori* plays an important role in multidrug resistance. World J Gastroenterol. 2008;14:5217–22.
26. Zhang Z, Liu ZQ, Zheng PY, Tang FA, Yang PC. Influence of efflux pump inhibitors on the multidrug resistance of *Helicobacter pylori*. World J Gastroenterol. 2010;16:1279–84.
27. Kim JM, Kim JS, Kim N, Kim YJ, Kim IY, Chee YJ, et al. Gene mutations of 23S rRNA associated with clarithromycin resistance in *Helicobacter pylori* strains isolated from Korean patients. J Microbiol Biotechnol. 2008;18:1584–9.
28. Lee JW, Kim SJ, Choi CW, Kim HJ, Kang DH, Kim HW, et al. Seven-day triple therapy is sufficient to eradicate infection caused by *Helicobacter pylori* without 23S rRNA point mutation. Medicine (Baltimore). 2021;100:e26133.
29. Stone GG, Shortridge D, Versalovic J, Beyer J, Flamm RK, Graham DY, et al. A PCR-oligonucleotide ligation assay to determine the prevalence of 23S rRNA gene mutations in clarithromycin-resistant *Helicobacter pylori*. Antimicrob Agents Chemother. 1997;41:712–4.
30. Cambau E, Allerheiligen V, Coulon C, Corbel C, Lascols C, Deforges L, et al. Evaluation of a new test, genotype HelicoDR, for molecular detection of antibiotic resistance in *Helicobacter pylori*. J Clin Microbiol. 2009;47:3600–7.
31. Lehours P, Siffre E, Megraud F. DPO multiplex PCR as an alternative to culture and susceptibility testing to detect *Helicobacter pylori* and its resistance to clarithromycin. BMC Gastroenterol. 2011;11:112.
32. Kwon YH, Jeon SW, Nam SY, Lee HS, Park JH. Efficacy of tailored therapy for *Helicobacter pylori* eradication based on clarithromycin resistance and survey of previous antibiotic exposure: a single-center prospective pilot study. Helicobacter. 2019;24:e12585.
33. Gweon TG, Kim JS, Kim BW. An economic modeling study of *Helicobacter pylori* eradication: comparison of dual priming oligonucleotide-based multiplex polymerase chain reaction and empirical treatment. Gut Liver. 2018;12:648–54.
34. Egli K, Wagner K, Keller PM, Risch L, Risch M, Bodmer T. Comparison of the diagnostic performance of qPCR, sanger sequencing, and whole-genome sequencing in determining clarithromycin and levofloxacin resistance in *Helicobacter pylori*. Front Cell Infect Microbiol. 2020;10:596371.
35. Binh TT, Shiota S, Suzuki R, Matsuda M, Trang TT, Kwon DH, et al. Discovery of novel mutations for clarithromycin resistance in *Helicobacter pylori* by using next-generation sequencing. J Antimicrob Chemother. 2014;69:1796–803.
36. Iwamoto A, Tanahashi T, Okada R, Yoshida Y, Kikuchi K, Keida Y, et al. Whole-genome sequencing of clarithromycin resistant *Helicobacter pylori* characterizes unidentified variants of multidrug resistant efflux pump genes. Gut Pathog. 2014;6:27.
37. Yonezawa H, Osaki T, Hanawa T, Kurata S, Ochiai K, Kamiya S. Impact of *Helicobacter pylori* biofilm formation on clarithromycin susceptibility and generation of resistance mutations. PLoS One. 2013;8:e73301.
38. Kocsis B, Gulyas D, Szabo D. Delafloxacin, finafloxacin, and zabofloxacin: novel fluoroquinolones in the antibiotic pipeline. Antibiotics (Basel). 2021;10:1506.
39. Lee JW, Kim N, Nam RH, Kim JM, Park JY, Lee SM, et al. High efficacy of finafloxacin on *Helicobacter pylori* isolates at pH 5.0 compared with that of other fluoroquinolones. Antimicrob Agents Chemother. 2015;59:7629–36.
40. Chang WL, Kao CY, Wu CT, Huang AH, Wu JJ, Yang HB, et al. Gemifloxacin can partially overcome quinolone resistance of *H. pylori* with *gyrA* mutation in Taiwan. Helicobacter. 2012;17:210–5.
41. Hsu PI, Chuah SK, Yamaoka Y, Wu DC. Cut-off value of clarithromycin resistance in the treatment of *Helicobacter pylori* infection: how low is low? Gut. 2024;73:374–5.

40

Amoxicillin

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Abstract

Treatment of *Helicobacter pylori* (*H. pylori*) is warranted and normally done with combination of antibiotics (such as amoxicillin, clarithromycin, or others) and a proton pump inhibitor. However, the eradication rate of legacy triple therapy has been decreasing because of the increase in resistance to clarithromycin. Likewise, treatment failure due to amoxicillin, a primary antimicrobial agent for *H. pylori* eradication, resistance has been noted since 20 years ago. Increasing levels of resistance to amoxicillin contribute to treatment failures, and higher levels of resistance are believed to be due to multiple genetic mutations. However, the exact mechanism of amoxicillin resistance and its contribution to eradication failure has not been fully elucidated.

Keywords

Amoxicillin · Antibiotic resistance
Eradication · *Helicobacter pylori*

40.1 Introduction

Helicobacter pylori (*H. pylori*) is a major cause of peptic ulcer disease and potentially stomach cancer [1–3]. Recent decades have seen the standardization of treatment for *H. pylori* eradication, and all of the worldwide consensus conferences have recommended the use of triple therapy consisting of a proton pump inhibitor (double dose) and two antibiotics, mainly amoxicillin (1 g b.i.d.) and clarithromycin (500 mg b.i.d.) or metronidazole (500 mg b.i.d.), as first-line therapy [4, 5]. However, this conventional triple therapy has been showing decreased eradication rate with geographic difference [6, 7]. It is important that treatment failures of *H. pylori* occur principally because of poor patient compliance or bacterial resistance [8]. Antibiotic resistance of *H. pylori* has been regarded to be mainly related with metronidazole and macrolide such as clarithromycin [9–16]. Amoxicillin is a β-lactam antibiotic and constitutes the primary *H. pylori* eradication regimen [17–19]. In the past, even until the last decade it was generally considered that amoxicillin resistance of *H. pylori* was absent or very rare. But currently, reports on the isolation of amoxicillin resistant *H. pylori* are increasing worldwide [7, 14, 20–23]. The objective of the present chapter is to review the prevalence of *H. pylori* resistance to amoxicillin, clinical importance, and the resistance mechanism of amoxicillin in *H. pylori* infection.

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40.2 Prevalence of *H. pylori* Resistance to Amoxicillin

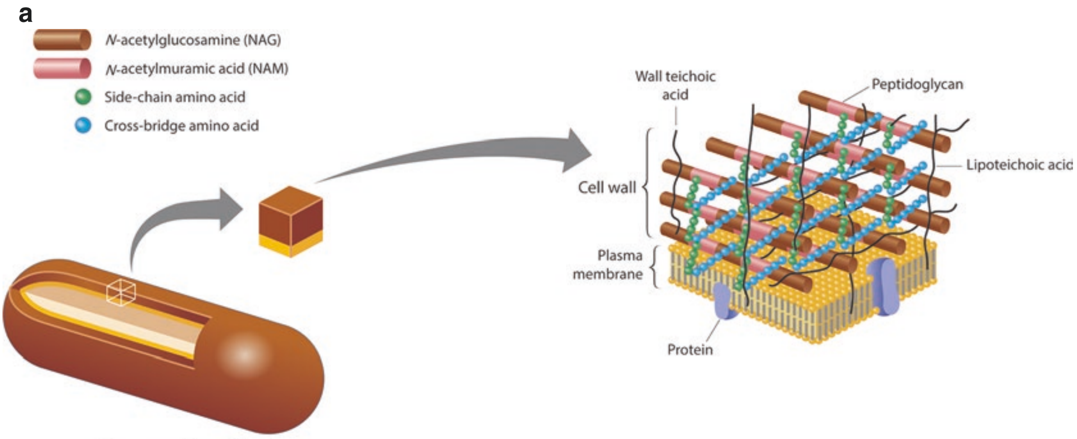
Until the end of the twentieth century, resistance to penicillins (e.g., amoxicillin) was very rare in *H. pylori*. By contrast, many other bacteria exhibit widespread resistance to these antibiotics (clarithromycin and metronidazole) [21, 24–26]. The prevalence of antibiotic resistance in various regions is correlated with the general use of antibiotics in the region and *H. pylori* strains have been divided into five major groups (east Asian type, south/central Asian type, Iberian/African type, and European type) according to geographical associations [6, 7, 27]. The most important factor resulting in antibiotic resistance is poor patient adherence to the drug regimen. However, the incidence of amoxicillin (minimal inhibitory concentration [MIC] ≥0.5 mg/L, respectively) in *H. pylori* seems to increase in geographic regions where these antibiotics can be obtained without prescription. The first indications of an increased incidence of amoxicillin resistance in *H. pylori* came from amoxicillin-resistant isolates obtained from dyspeptic patients in Italy and the USA [28]. Amoxicillin resistance of these isolates is not stable, as it is lost upon freezing at −80 °C. Thus, these isolates are referred to as amoxicillin-tolerant rather than amoxicillin-resistant [28]. Later, stable Amoxicillin-resistant *H. pylori* isolates were obtained from dyspeptic patients living in different geographic regions [29, 30].

However, most of the surveys previously reported that resistance to amoxicillin is either null or less than 1%, indicating that it is not yet a problem [7]. In Europe, available data from two studies enrolling 599 patients found a prevalence rate < 1% (3/599, 0.5%; 95% confidence interval [CI], 0.06–1.06). Recent study reported that the rate of amoxicillin resistance was 0.7% in 1893 European adults [31]. According to systemic review, the primary amoxicillin resistance was detected in 184 out of 1640 tested patients (11.2%; 95% CI, 9.6–12.7) [21]. In Europe, available data from two studies enrolling 599 patients found a prevalence rate < 1% (3/599, 0.5%; 95% CI, 0.06–1.06) [21]. On the contrary, conflicting data were reported in two African studies [21]. Indeed, amoxicillin resistance was absent in a study from Senegal enrolling 40 patients, while an astonishingly high prevalence was reported in Cameroon (113/132, 85.6%; 95% CI, 76.9–91.5) [32, 33]. Similarly, the prevalence of amoxicillin resistance widely varies in Asian countries, ranging from 0% in 61 patients in Japan, 8.8% (10/113; 95% CI, 3.6–14.0) in Korea, and 36.1% (48/133; 95% CI, 27.9–44.2) in Taiwan, although another study performed in Taiwan found a prevalence as low as 0.9% (2/210; 95% CI, 0.3–2.2) [14, 21, 34–36]. According to another Japanese study, no amoxicillin resistance strains were detected in the strains isolated between 1985 and 1996, while the rate of resistance was determined to be 1.1%, 2.1%, 5.4%, 5.6%, 0%, 8.8%, and 1.5% each year, respectively, from 1997 to 2003 [37]. The percentage of resistance to amoxicillin strains increased from 2000 to 2003. The total eradication rate of *H. pylori* in the patients who showed amoxicillin-resistant strains was 71.0% (44/62) [37]. In Korea, the resistance to clarithromycin, tetracycline, ciprofloxacin, and levofloxacin increased during the period of 2007–2009 compared to 2003–2005 [14, 38]. However, amoxicillin resistance slightly decreased from 6.1% to 4.8% [38]. In a recent systematic review that analyzed antibiotic resistance across 178 studies, comprising 66,142 isolates from 65 countries, primary resistance to amoxicillin was found to be less than 10% worldwide, except in the Eastern Mediterranean region (14%) [39].

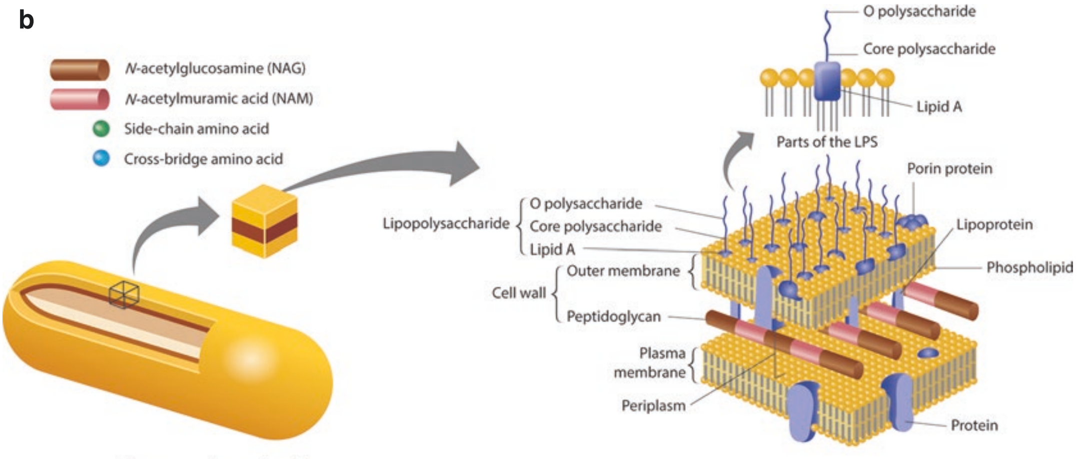
40.3 The Role and Resistance Mechanism of Amoxicillin in Eradication of *H. pylori*

40.3.1 Target of ß-Lactam Antibiotics in *H. pylori*

The cell wall of Gram-negative bacteria consists of a number of layers, differing greatly in their chemical composition. The inner most membrane is the cytoplasmic membrane followed by a thin peptidoglycan layer, different in composition and thickness from the Gram-positive peptidoglycan layer [40] (Fig. 40.1). Outside of the peptidoglycan layer is the outer membrane which contains lipopolysaccharide, consisting of lipid A, core polysaccharide, and O antigen. The peptidoglycan layer consists of *N*-acetyl glucosamine and *N*-acetyl muramic acid with a pentapeptide side chain. These units are cross-linked, forming a peptide bond between $_D$-alanine on one chain and diaminopimelic acid on the other chain in Gram-negative bacteria. This cross-linking function is performed by transpeptidase enzymes known as penicillin binding proteins (PBPs). The transpeptidase domains of these enzymes utilize an active site serine to perform the catalytic reaction [41]. PBPs also have transglycosylase activity responsible for peptidoglycan polymerization and insertion into preexisting cell wall, especially during new cell growth [42, 43]. β-lactam antibiotics are bacteriocidal and inhibit the synthesis of new peptidoglycan layer in actively dividing cells. Transpeptidase enzymes utilize an active


a
N-acetylglucosamine (NAG)
N-acetylmuramic acid (NAM)
Side-chain amino acid
Cross-bridge amino acid
Wall teichoic acid
Peptidoglycan
Lipoteichoic acid
Cell wall
Plasma membrane
Protein

Gram-positive cell wall


b
N-acetylglucosamine (NAG)
N-acetylmuramic acid (NAM)
Side-chain amino acid
Cross-bridge amino acid
O polysaccharide
Core polysaccharide
Lipid A
Parts of the LPS
Porin protein
Lipopolysaccharide
O polysaccharide
Core polysaccharide
Lipid A
Lipoprotein
Phospholipid
Outer membrane
Cell wall
Peptidoglycan
Plasma membrane
Periplasm
Protein

Gram-negative cell wall

Fig. 40.1 The structures of Gram-positive and Gram-negative cell wall. (**a**) In the Gram-positive bacteria (those that retain the purple crystal violet dye when subjected to the Gram-staining procedure), the cell wall consists of several layers of peptidoglycan. Running perpendicular to the peptidoglycan sheets is a group of molecules called teichoic acids which are unique to the Gram-positive cell wall. (**b**) In the Gram-negative bacteria (which do not retain the crystal violet), the cell wall is composed of a single layer of peptidoglycan surrounded by a membranous structure called the outer membrane. The outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS or endotoxin), which is toxic to animals. In Gram-negative bacteria, the outer membrane is usually thought of as part of the cell wall. (Adapted from Gerard et al. [40])

site serine and perform their catalytic cycle by way of an acylation/deacylation pathway. β-lactam antibiotics efficiently inhibit the bacterial transpeptidases, therefore these enzymes are often termed penicillin-binding proteins or PBPs. They are able to do this owing to the similarity of the b-lactam moiety with the D-alanine–D-alanine substrate. In the presence of the antibiotic, the transpeptidases form a lethal covalent penicilloyl–enzyme complex that serves to block the normal transpeptidation reaction. This results in weakly cross-linked peptidoglycan, which makes the growing bacteria highly susceptible to cell lysis and death [41]. Amoxicillin is extended spectrum penicillin and clavunate potassium is a β-lactamase inhibitor [44]. The molecular formula is C16H19N3O5S and the molecular weight is 419.45 (Fig. 40.2). Amoxicillin also acts on cell wall PBPs and may play role as an important stress factor for the morphologic changes by expression induction of some peptidase enzymes genes that result in modification of the cell wall compositions in coccoid forms. Amoxicillin had the greatest in vitro bactericidal effect on bacillary forms of *H. pylori*; however, in 2× MIC it had no bactericidal effect on coccoids [45]. In addition to the potent in vitro effect of amoxicillin against *H. pylori*, this antibiotic has a little in vivo bactericidal effect [46]. Transition to coccoid forms correlates with accumulation of the *N*-acetyl-D-glucosaminyl-β(1,4)-*N*-acetylmuramyl-L-Ala-D-Glu (GM-dipeptide) motif. This modification may have a role in resistance to bactericidal agents such as amoxicillin that act on the cell wall such as amoxicillin [47].

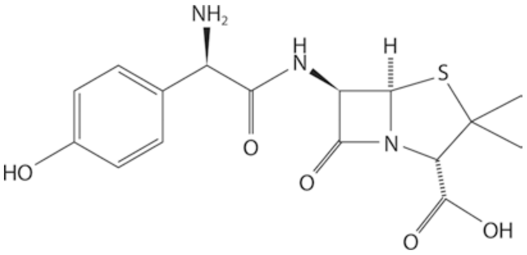


Fig. 40.2 The molecular formula of amoxicillin (2*S*,5*R*,6*R*)-6-{[(2*R*)-2-amino-2-(4-hydroxyphenyl)-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-24-carboxylic acid

40.3.2 The Resistance Mechanism of Amoxicillin

Among β-lactams, amoxicillin is frequently used for treating *H. pylori* infection. ß-lactams are rendered inactive against bacteria by way of three primary mechanisms of resistance (Fig. 40.3). The most common mechanism is the production of enzymes that degrade or modify the antibiotic before it can reach the appropriate target site. In this case, the ß-lactamase family of enzymes degrade ß-lactam antibiotics and are found widely disseminated among Gram-positive and Gram-negative bacteria. The second mechanism is alteration of the antibiotic target site. In this case, the ß-lactam-resistant cell-wall transpeptidases perform this role; this is now a major cause of resistance in several pathogens including the problematic Gram-positive *Staphylococcal* and *Streptococcal* species. The final mechanism is inhibition of access of the antibiotic to the target by way of altered permeability or forced efflux. For example, this can be performed by the Mex A,B–OprM antibiotic efflux pump, which is a major cause of resistance in Pseudomonas and in other pathogenic Gram-negative species [41]. In *H. pylori*, however, β-lactamase activity has not been established and resistance seems to be mainly mediated by alterations to PBPs. The PBPs are a set of enzymes involved in the synthesis of the peptidoglycan layer of the bacterial cell wall and include transpeptidases, transglycosylases, endopeptidases, and carboxypeptidases [48, 49]. All conventional PBPs contain characteristic penicillin-binding protein motifs (PBP-motifs); SXXK, SXN, and KTG (X is a variable amino acid residue) [50]. In *H. pylori*, nine putative PBPs have been identified; Four PBPs have been identified using labeled antibiotics: DeLoney et al. [20] reported the following molecular masses of four major PBPs in *H. pylori* ATCC 43579: 66, 63, 60, and 47 kDa. The molecular mass of a small PBP was reported in the range of 30–32 kDa by Dore et al. [28] (named PBP D) and Krishnamurthy et al. [51] (named PBP4). They also identified three high-molecular-mass PBPs (PBPs 1, 2, and 3) from *H. pylori* 84–183 in the range of 66 to 55 kDa and indicated that these

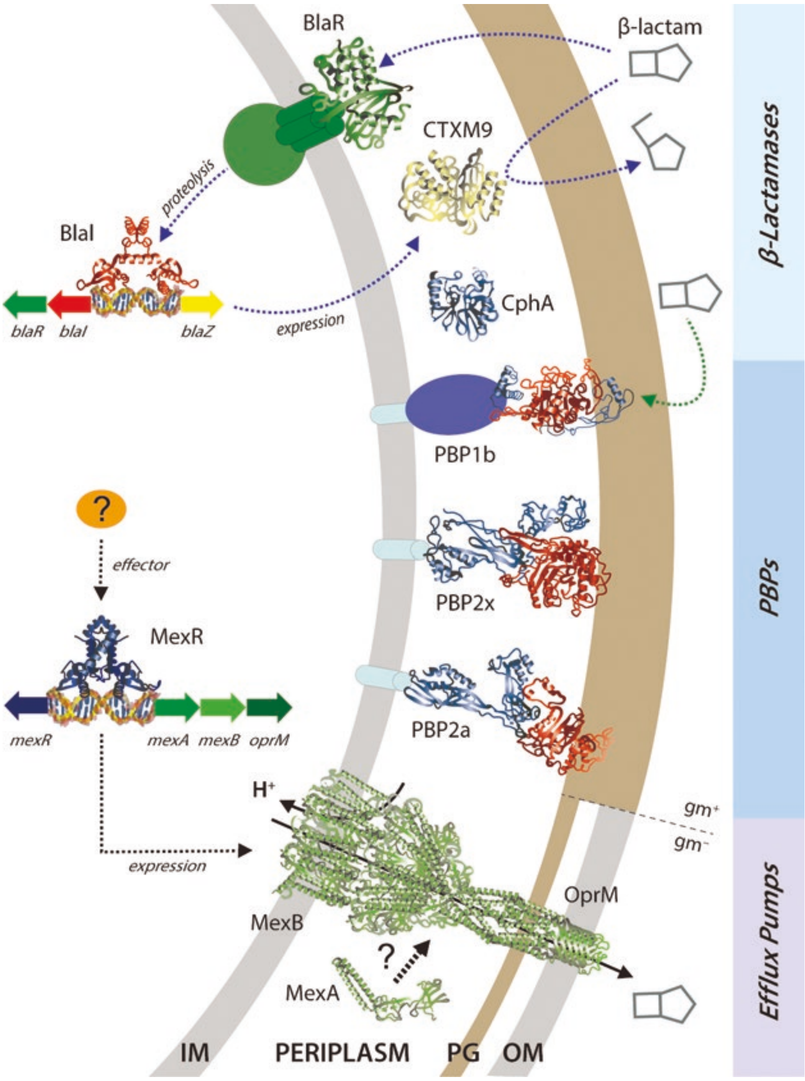

BlaR
β-lactam
CTXM9
proteolysis
BlaI
blaR
blaI
blaZ
expression
CphA
β-Lactamases
PBP1b
?
effector
MexR
PBP2x
PBPs
PBP2a
mexR
mexA
mexB
oprM
H+
gm+
gm-
expression
OprM
MexB
?
MexA
Efflux Pumps
IM
PERIPLASM
PG
OM

Fig. 40.3 The mechanism of β-lactam antibiotic. (Adapted from Wilke et al. [41]). *gm* +/− Gram-positive/negative, *IM* inner membrane, *OM* outer membrane, *PG* peptidoglycan. CTXM9 and CphA are shown as representatives of the >40 available serine and metallo-β-lactamase structures

PBPs corresponded to PBPs A, B, and C [51], previously described by Ikeda et al. [52]. Other PBPs in *H. pylori* were recently identified by Harris et al. [50] at 72, 62, 54, 50, 44, 33.5, 30.5, and 28 kDa. Gram-negative bacteria have porin proteins in their outer membrane which facilitate the transport of hydrophilic solutes. In previously obtained clinically resistant strains, changes in PBP1 alone do not account for all of the resistance observed. In addition to changes in PBPs, reduced membrane permeability and active efflux have been suggested as mechanisms contributing to higher resistance to β-lactam drugs. A change in the property of PBP, either a decreased affinity for amoxicillin [53] or point mutation in the *pbp1* gene [54], is the main mechanism that affects the amoxicillin resistance of *H. pylori* [55]. PBP1 has been studied most extensively and has been shown to play a significant role in conferring β-lactam resistance to *H. pylori* [56]. A careful review of the amino acid changes in *pbp1* in amoxicillin-resistant strains from previous publications has led to our hypothesis that only a few of amino acid substitutions can alter amoxicillin binding Ser414Arg [57–61], Thr438Met [56], Phe473Leu [62], Ser543Arg [55, 62], Thr556Tyr [55, 58, 63, 64], and Asn562Tyr [55, 58, 60, 61, 63] (Table 40.1). These amino acid substitutions

Table 40.1 Review of previously identified mutations in PBP1 from amoxicillin-resistant isolate

Author	V 16 I	V 45 I	P 372 S	S 402 G	E 406 A	S 414 R	S 414 A	S 417 T	T 438 M	Y 484 C	E 506 A	M 515 I	S 517 T	D 535 N	T 541 I	S 543 R	T 556 S	N 562 Y	A 562 T	T 593 A	G 595 S	P 600 T
Co and Schiller [56]									O													
Gerrits et al. [22]				O	O	O		O														
Kwon et al. [55]											O	O	O	O		O	O	O				
Matteo et al. [65]																	O					
Okamoto et al. [63]																	O	O		O		
Paul et al. [59]						O				O					O		O	O				O
Rimbara et al. [60]						O												O				
Qureshi et al. [62, 68]			O						O							O	O	O				
Kim and Kim [69]	O	O					O												O	O	O	

PBP penicillin-binding protein

have appeared either as a single change with corresponding increased amoxicillin resistance [56, 57, 65] or as parts of clusters of changes [55, 58, 60, 62–64]. In all cases the authors obtained transformants with increased MICs but not higher than 0.5–2 mg/L. However, sequencing of *pbp1A* in these different experiments showed different mutations associated with resistance. Dore et al. [28] reported that the 30–32 kDa PBP (PBP D) band observed in amoxicillin-susceptible strains was not detected in resistant strains from various sources and proposed that PBP D may play a role in the amoxicillin-resistant phenotype of *H. pylori*. They did not discuss whether the disappearance of the band is due to a lack of the PBP D protein or to reduced affinity for [3H] benzylpenicillin because of a change in PBP D. Thus, the contributions of these individual mutations to amoxicillin resistance are not clear, since several other mutations are also present in *pbp1*. Mutational changes in other PBPs are suspected to facilitate and enhance primary resistance owing to PBP1A alterations [66].

By culturing an amoxicillin-susceptible strain with increasingly higher concentrations of amoxicillin, it was also possible to obtain strains with MICs of 4–8 mg/L which exhibited a decreased affinity for this drug [67]. Amoxicillin resistant *H. pylori* strains accumulated less [^{14}C]-penicillin G compared with amoxicillin-susceptible strains, both in the presence and absence of CCCP, thus excluding the role of an active efflux mechanism [55, 67]. This suggests that at least in some strains, β-lactam resistance in *H. pylori* is partly due to an increased diffusional barrier, an effect that could be explained by changes in outer membrane proteins. By comparing an in vitro selected amoxicillin-resistant strain with its amoxicillin-sensitive parent strain, it has been determined that both changes in PBP1 and Hop Proteins B and C also seemed to contribute to resistance [55] and the combined effects of amino acid changes occurring in PBP1, HopC, HefC, HofH, and possibly PBP2, recently [68].

However, most of these individual mutations that contributed to amoxicillin resistance were evaluated by comparing the amoxicillin-resistant *H. pylori* strains with reference strains, or using site-directed mutagenesis to create mutations individually in *pbp1* and transformed each altered *pbp1* into amoxicillin-sensitive strain to monitor its effect on the MIC of *H. pylori* amoxicillin, in vitro. Thus, the clinical significance of these mutations to affect amoxicillin resistance and failure of eradication was doubtful.

40.4 Conclusions

Reports on the incidence of amoxicillin resistance are still conflicting and not investigated, yet. If amoxicillin resistance in *H. pylori* would further increase, this would pose the serious problem of reduced efficacy of amoxicillin-containing regimens. Based on previous studies, the complexity of several mechanisms (*pbp* gene mutations, membrane permeability alterations, efflux pumps, etc.) was related with amoxicillin resistance in *H. pylori* infection. Moreover, these mutations would affect the failure of *H. pylori* eradication with amoxicillin-containing therapy, which is another problem. Many clinical and experimental data would be needed to understand the effect and resistance of amoxicillin in *H. pylori*.

References

1. Malfertheiner P, Chan FK, McColl KE. Peptic ulcer disease. Lancet. 2009;374:1449–61.
2. Correa P, Piazuelo MB. The gastric precancerous cascade. J Dig Dis. 2012;13:2–9.
3. Plummer M, Franceschi S, Vignat J, Forman D, de Martel C. Global burden of gastric cancer attributable to *Helicobacter pylori*. Int J Cancer. 2015;136:487–90.
4. Lam SK, Talley NJ. Report of the 1997 Asia Pacific consensus conference on the management of *Helicobacter pylori* infection. J Gastroenterol Hepatol. 1998;13:1–12.
5. Vakil N, Megraud F. Eradication therapy for *Helicobacter pylori*. Gastroenterology. 2007;133:985–1001.
6. Boyanova L, Mitov I. Geographic map and evolution of primary *Helicobacter pylori* resistance to antibacterial agents. Expert Rev Anti-Infect Ther. 2010;8:59–70.
7. Megraud F. *H pylori* antibiotic resistance: prevalence, importance, and advances in testing. Gut. 2004;53:1374–84.

8. Lind T, Mégraud F, Unge P, Bayerdörffer E, O'Morain C, Spiller R, et al. The MACH2 study: role of omeprazole in eradication of *Helicobacter pylori* with 1-week triple therapies. Gastroenterology. 1999;116:248–53.
9. De Francesco V, Margiotta M, Zullo A, Hassan C, Troiani L, Burattini O, et al. Clarithromycin-resistant genotypes and eradication of *Helicobacter pylori*. Ann Intern Med. 2006;144:94–100.
10. Agudo S, Alarcón T, Cibrelus L, Urruzuno P, Martinez MJ, López-Brea M. High percentage of clarithromycin and metronidazole resistance in *Helicobacter pylori* clinical isolates obtained from Spanish children. Rev Esp Quimioter. 2009;22:88–92.
11. Liu Z, Shen J, Zhang L, Shen L, Li Q, Zhang B, et al. Prevalence of A2143G mutation of *H. pylori*-23S rRNA in Chinese subjects with and without clarithromycin use history. BMC Microbiol. 2008;8:81.
12. De Francesco V, Zullo A, Ierardi E, Giorgio F, Perna F, Hassan C, et al. Phenotypic and genotypic *Helicobacter pylori* clarithromycin resistance and therapeutic outcome: benefits and limits. J Antimicrob Chemother. 2010;65:327–32.
13. Kim JM, Kim JS, Kim N, Kim YJ, Kim IY, Chee YJ, et al. Gene mutations of 23S rRNA associated with clarithromycin resistance in *Helicobacter pylori* strains isolated from Korean patients. J Microbiol Biotechnol. 2008;18:1584–9.
14. Kim N, Kim JM, Kim CH, Park YS, Lee DH, Kim JS, et al. Institutional difference of antibiotic resistance of *Helicobacter pylori* strains in Korea. J Clin Gastroenterol. 2006;40:683–7.
15. Binh TT, Suzuki R, Kwon DH, Yamaoka Y. Complete genome sequence of a metronidazole-resistant *Helicobacter pylori* strain. Genome Announc. 2015;3:e00051–15.
16. Binh TT, Suzuki R, Trang TT, Kwon DH, Yamaoka Y. Search for novel candidate mutations for metronidazole resistance in *Helicobacter pylori* using next-generation sequencing. Antimicrob Agents Chemother. 2015;59:2343–8.
17. Gisbert JP, Calvet X. Review article: the effectiveness of standard triple therapy for *Helicobacter pylori* has not changed over the last decade, but it is not good enough. Aliment Pharmacol Ther. 2011;34:1255–68.
18. Fischbach LA, Goodman KJ, Feldman M, Aragaki C. Sources of variation of *Helicobacter pylori* treatment success in adults worldwide: a meta-analysis. Int J Epidemiol. 2002;31:128–39.
19. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of *Helicobacter pylori* infection—the Maastricht IV/ Florence consensus report. Gut. 2012;61:646–64.
20. DeLoney CR, Schiller NL. Competition of various beta-lactam antibiotics for the major penicillin-binding proteins of *Helicobacter pylori*: antibacterial activity and effects on bacterial morphology. Antimicrob Agents Chemother. 1999;43:2702–9.
21. De Francesco V, Giorgio F, Hassan C, Manes G, Vannella L, Panella C, et al. Worldwide *H. pylori* antibiotic resistance: a systematic review. J Gastrointestin Liver Dis. 2010;19:409–14.
22. Gerrits MM, Godoy AP, Kuipers EJ, Ribeiro ML, Stoof J, Mendonca S, et al. Multiple mutations in or adjacent to the conserved penicillin-binding protein motifs of the penicillin-binding protein 1A confer amoxicillin resistance to *Helicobacter pylori*. Helicobacter. 2006;11:181–7.
23. Debets-Ossenkopp YJ, Herscheid AJ, Pot RG, Kuipers EJ, Kusters JG, Vandenbroucke-Grauls CM. Prevalence of *Helicobacter pylori* resistance to metronidazole, clarithromycin, amoxycillin, tetracycline and trovafloxacin in The Netherlands. J Antimicrob Chemother. 1999;43:511–5.
24. Versalovic J, Shortridge D, Kibler K, Griffy MV, Beyer J, Flamm RK, et al. Mutations in 23S rRNA are associated with clarithromycin resistance in *Helicobacter pylori*. Antimicrob Agents Chemother. 1996;40:477–80.
25. van Zwet AA, de Boer WA, Schneeberger PM, Weel J, Jansz AR, Thijs JC. Prevalence of primary *Helicobacter pylori* resistance to metronidazole and clarithromycin in The Netherlands. Eur J Clin Microbiol Infect Dis. 1996;15:861–4.
26. Vasquez A, Valdez Y, Gilman RH, McDonald JJ, Westblom TU, Berg D, et al. Metronidazole and clarithromycin resistance in *Helicobacter pylori* determined by measuring MICs of antimicrobial agents in color indicator egg yolk agar in a miniwell format. The Gastrointestinal Physiology Working Group of Universidad Peruana Cayetano Heredia and the Johns Hopkins University. J Clin Microbiol. 1996;34:1232–4.
27. Wu W, Yang Y, Sun G. Recent insights into antibiotic resistance in *Helicobacter pylori* eradication. Gastroenterol Res Pract. 2012;2012:723183.
28. Dore MP, Graham DY, Sepulveda AR. Different penicillin-binding protein profiles in amoxicillin-resistant *Helicobacter pylori*. Helicobacter. 1999;4:154–61.
29. Meyer JM, Silliman NP, Wang W, Siepman NY, Sugg JE, Morris D, et al. Risk factors for *Helicobacter pylori* resistance in the United States: the surveillance of *H. pylori* antimicrobial resistance partnership (SHARP) study, 1993–1999. Ann Intern Med. 2002;136:13–24.
30. Glupczynski Y, Mégraud F, Lopez-Brea M, Andersen LP. European multicentre survey of in vitro antimicrobial resistance in *Helicobacter pylori*. Eur J Clin Microbiol Infect Dis. 2001;20:820–3.
31. Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, et al. *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut. 2013;62:34–42.
32. Ndip RN, Malange Takang AE, Ojongokpoko JE, Luma HN, Malongue A, Akoachere JF, et al. *Helicobacter pylori* isolates recovered from gastric biopsies of patients with gastro-duodenal pathologies in Cameroon: current status of antibiogram. Tropical Med Int Health. 2008;13:848–54.

33. Seck A, Mbengue M, Gassama-Sow A, Diouf L, Ka MM, Boye CS. Antibiotic susceptibility of *Helicobacter pylori* isolates in Dakar, Senegal. J Infect Dev Ctries. 2009;3:137–40.
34. Kobayashi I, Murakami K, Kato M, Kato S, Azuma T, Takahashi S, et al. Changing antimicrobial susceptibility epidemiology of *Helicobacter pylori* strains in Japan between 2002 and 2005. J Clin Microbiol. 2007;45:4006–10.
35. Hung KH, Sheu BS, Chang WL, Wu HM, Liu CC, Wu JJ. Prevalence of primary fluoroquinolone resistance among clinical isolates of *Helicobacter pylori* at a University Hospital in Southern Taiwan. Helicobacter. 2009;14:61–5.
36. Hu CT, Wu CC, Lin CY, Cheng CC, Su SC, Tseng YH, et al. Resistance rate to antibiotics of *Helicobacter pylori* isolates in eastern Taiwan. J Gastroenterol Hepatol. 2007;22:720–3.
37. Watanabe K, Tanaka A, Imase K, Tokunaga K, Sugano H, Kai A, et al. Amoxicillin resistance in *Helicobacter pylori*: studies from Tokyo, Japan from 1985 to 2003. Helicobacter. 2005;10:4–11.
38. Hwang TJ, Kim N, Kim HB, Lee BH, Nam RH, Park JH, et al. Change in antibiotic resistance of *Helicobacter pylori* strains and the effect of A2143G point mutation of 23S rRNA on the eradication of *H. pylori* in a single center of Korea. J Clin Gastroenterol. 2010;44:536–43.
39. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of antibiotic resistance in *Helicobacter pylori*: a systematic review and meta-analysis in World Health Organization regions. Gastroenterology. 2018;155:1372–82.e17.
40. Tortora GJ, Funke BR, Case CL. Microbiology: an introduction. 12th ed. New York: Pearson Education, Inc.; 2016. p. p82.
41. Wilke MS, Lovering AL, Strynadka NC. Beta-lactam antibiotic resistance: a current structural perspective. Curr Opin Microbiol. 2005;8:525–33.
42. Goffin C, Ghuysen JM. Multimodular penicillin-binding proteins: an enigmatic family of orthologs and paralogs. Microbiol Mol Biol Rev. 1998;62:1079–93.
43. Born P, Breukink E, Vollmer W. In vitro synthesis of cross-linked murein and its attachment to sacculi by PBP1A from *Escherichia coli*. J Biol Chem. 2006;281:26985–93.
44. Handsfield HH, Clark H, Wallace JF, Holmes KK, Turck M. Amoxicillin, a new penicillin antibiotic. Antimicrob Agents Chemother. 1973;3:262–5.
45. Faghri J, Poursina F, Moghim S, Zarkesh Esfahani H, Nasr Esfahani B, Fazeli H, et al. Morphological and bactericidal effects of different antibiotics on *Helicobacter pylori*. Jundishapur J Microbiol. 2014;7:e8704.
46. Berry V, Jennings K, Woodnutt G. Bactericidal and morphological effects of amoxicillin on *Helicobacter pylori*. Antimicrob Agents Chemother. 1995;39:1859–61.
47. Costa K, Bacher G, Allmaier G, Dominguez-Bello MG, Engstrand L, Falk P, et al. The morphological transition of *Helicobacter pylori* cells from spiral to coccoid is preceded by a substantial modification of the cell wall. J Bacteriol. 1999;181:3710–5.
48. Blumberg PM, Strominger JL. Interaction of penicillin with the bacterial cell: penicillin-binding proteins and penicillin-sensitive enzymes. Bacteriol Rev. 1974;38:291–335.
49. Ghuysen JM. Penicillin-binding proteins. Wall peptidoglycan assembly and resistance to penicillin: facts, doubts and hopes. Int J Antimicrob Agents. 1997;8:45–60.
50. Harris AG, Hazell SL, Netting AG. Use of digoxigenin-labelled ampicillin in the identification of penicillin-binding proteins in *Helicobacter pylori*. J Antimicrob Chemother. 2000;45:591–8.
51. Krishnamurthy P, Parlow MH, Schneider J, Burroughs S, Wickland C, Vakil NB, et al. Identification of a novel penicillin-binding protein from *Helicobacter pylori*. J Bacteriol. 1999;181:5107–10.
52. Ikeda F, Yokota Y, Mine Y, Tatsuta M. Activity of cefixime against *Helicobacter pylori* and affinities for the penicillin-binding proteins. Antimicrob Agents Chemother. 1990;34:2426–8.
53. Dore MP, Osato MS, Realdi G, Mura I, Graham DY, Sepulveda AR. Amoxycillin tolerance in *Helicobacter pylori*. J Antimicrob Chemother. 1999;43:47–54.
54. van Zwet AA, Vandenbroucke-Grauls CM, Thijs JC, van der Wouden EJ, Gerrits MM, Kusters JG. Stable amoxicillin resistance in *Helicobacter pylori*. Lancet. 1998;352:1595.
55. Kwon DH, Dore MP, Kim JJ, Kato M, Lee M, Wu JY, et al. High-level beta-lactam resistance associated with acquired multidrug resistance in *Helicobacter pylori*. Antimicrob Agents Chemother. 2003;47:2169–78.
56. Co EM, Schiller NL. Resistance mechanisms in an in vitro-selected amoxicillin-resistant strain of *Helicobacter pylori*. Antimicrob Agents Chemother. 2006;50:4174–6.
57. Gerrits MM, Schuijffel D, van Zwet AA, Kuipers EJ, Vandenbroucke-Grauls CM, Kusters JG. Alterations in penicillin-binding protein 1A confer resistance to beta-lactam antibiotics in *Helicobacter pylori*. Antimicrob Agents Chemother. 2002;46:2229–33.
58. Gerrits MM, van Vliet AH, Kuipers EJ, Kusters JG. *Helicobacter pylori* and antimicrobial resistance: molecular mechanisms and clinical implications. Lancet Infect Dis. 2006;6:699–709.
59. Paul R, Postius S, Melchers K, Schäfer KP. Mutations of the *Helicobacter pylori* genes *rdxA* and *pbp1* cause resistance against metronidazole and amoxicillin. Antimicrob Agents Chemother. 2001;45:962–5.
60. Rimbara E, Noguchi N, Kawai T, Sasatsu M. Correlation between substitutions in penicillin-binding protein 1 and amoxicillin resistance in *Helicobacter pylori*. Microbiol Immunol. 2007;51:939–44.
61. Kim JM. Antibiotic resistance of *Helicobacter pylori* isolated from Korean patients. Korean J Gastroenterol. 2006;47:337–49.

62. Qureshi NN, Morikis D, Schiller NL. Contribution of specific amino acid changes in penicillin binding protein 1 to amoxicillin resistance in clinical *Helicobacter pylori* isolates. Antimicrob Agents Chemother. 2011;55:101–9.
63. Okamoto T, Yoshiyama H, Nakazawa T, Park ID, Chang MW, Yanai H, et al. A change in PBP1 is involved in amoxicillin resistance of clinical isolates of *Helicobacter pylori*. J Antimicrob Chemother. 2002;50:849–56.
64. Tseng YS, Wu DC, Chang CY, Kuo CH, Yang YC, Jan CM, et al. Amoxicillin resistance with beta-lactamase production in *Helicobacter pylori*. Eur J Clin Investig. 2009;39:807–12.
65. Matteo MJ, Granados G, Olmos M, Wonaga A, Catalano M. *Helicobacter pylori* amoxicillin heteroresistance due to point mutations in PBP-1A in isogenic isolates. J Antimicrob Chemother. 2008;61:474–7.
66. Hu Y, Zhang M, Lu B, Dai J. *Helicobacter pylori* and antibiotic resistance, a continuing and intractable problem. Helicobacter. 2016;21:349–63.
67. DeLoney CR, Schiller NL. Characterization of an in vitro-selected amoxicillin-resistant strain of *Helicobacter pylori*. Antimicrob Agents Chemother. 2000;44:3368–73.
68. Qureshi NN, Gallaher B, Schiller NL. Evolution of amoxicillin resistance of *Helicobacter pylori* in vitro: characterization of resistance mechanisms. Microb Drug Resist. 2014;20:509–16.
69. Kim BJ, Kim JG. Substitutions in penicillin-binding protein 1 in amoxicillin-resistant *Helicobacter pylori* strains isolated from Korean patients. Gut Liver. 2013;7:655–60.

Fluoroquinolone

41

Jung Won Lee

Abstract

Fluoroquinolone antibiotics demonstrate excellent antimicrobial efficacy in various clinical fields. Especially, levofloxacin and moxifloxacin have antimicrobial efficacy against *Helicobacter pylori* (*H. pylori*). They have been used effectively for *H. pylori* eradication with fewer side effects instead of bismuth quadruple therapy. Unfortunately, the recent rapid rise in resistance has made the prospect of fluoroquinolone-containing therapy unfavorable. The increase of resistance has become a significant limitation for providing effective eradication result. The main antimicrobial mechanism of fluoroquinolone is the interruption of DNA replication by interfering with DNA gyrase and topoisomerase IV. Resistance of fluoroquinolone antibiotics was derived from the mutation of *gyrA* which encodes DNA gyrase A, especially point mutation of Asn-87. However, novel fluoroquinolones show better efficacy as to overcome the resistance caused by *gyrA* mutation. Epidemiologic study for point mutation of resistance gene might be helpful for the development of molecular diagnostic tool and novel fluoroquinolone agent. The development of novel molecular methods for rapid determination of fluoroquinolone resistance could be the key to overcoming resistance.

Keywords

Helicobacter pylori · Antibiotics · Resistance Fluoroquinolone

41.1 Introduction

Triple therapy containing a high-dose proton pump inhibitor (PPI), amoxicillin with clarithromycin, the so-called legacy triple therapy, has been used for first-line eradication treatment for *Helicobacter pylori* (*H. pylori*) infection. Because of the high prevalence of metronidazole resistance, clarithromycin-containing therapy has been the mainstream of triple therapy. Unfortunately, the eradication rate of legacy triple therapy has been decreasing progressively by clarithromycin resistance. There has been urgent need to find alternative antimicrobial agent [1, 2].

Quinolones antimicrobial agents have been good alternative agent with considerable efficacy. The quinolones are a family of synthetic antibiotics developed at 1960s. The initial quinolone agents merely have limited indications due to their limited efficacy. At 1980s, quinolone antibiotics evolved to fluoroquinolones by attached fluorine bond. This structural modification provided significantly better efficacy to quinolone

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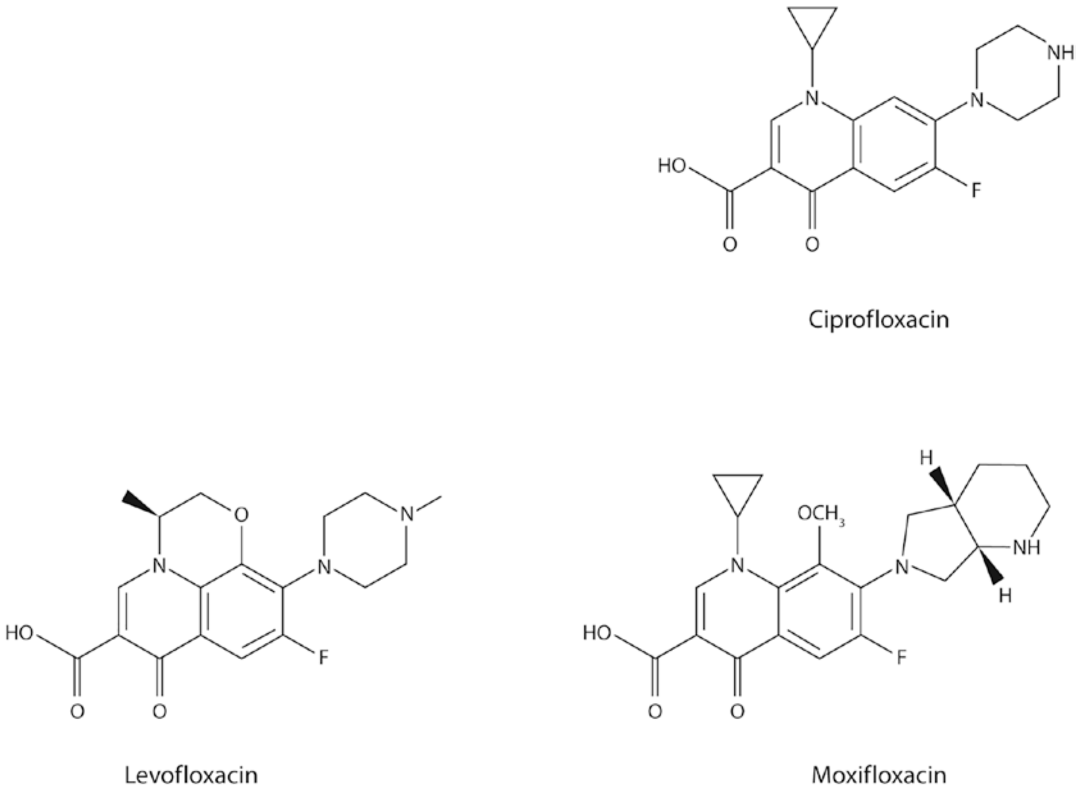

Fig. 41.1 Molecular structure of fluoroquinolone antibiotics

antibiotics. Representative second-generation quinolone is ciprofloxacin. Ciprofloxacin shows excellent antimicrobial efficacy especially to Gram-negative bacteria. After ciprofloxacin, next-generation fluoroquinolones such as levofloxacin and moxifloxacin were developed with more extended efficacy even to Gram-positive bacteria (Fig. 41.1). These next-generation fluoroquinolones have excellent bioavailability—about ≥90%—and their efficacy could be easily achieved by oral route. Adverse events are rare and not a major problem except in children. Levofloxacin and moxifloxacin provided acceptable antimicrobial efficacy compared to ciprofloxacin, especially for *H. pylori*. For this reason, many investigators performed studies on fluoroquinolone-containing eradication treatments, competitively. However, resistance of fluoroquinolone to *H. pylori* has increased in association with the rapid increase in fluoroquinolone prescription for other diseases treatment, such as respiratory and urinary tract diseases, and has become considerable limitation for maintaining efficacy as *H. pylori* eradication therapy [2, 3].

From this background, we describe mechanism of action, epidemiology, diagnosis, and resistance mechanism to fluoroquinolone in this chapter to provide updated clinical implication and perspectives for more effective *H. pylori* eradication therapy using fluoroquinolones.

41.2 Mechanism of Action of Fluoroquinolone

The main role of gyrase and topoisomerase was negative supercoiling by cutting DNA double helix during the initial phase of DNA replication. The mechanism of fluoroquinolone is the interruption of DNA replication by interfering with

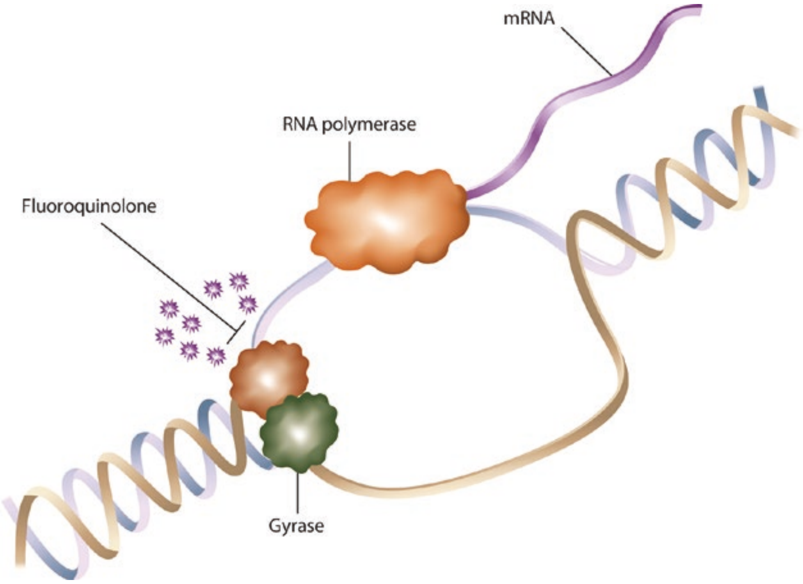

Fig. 41.2 Mechanism of action of fluoroquinolones

DNA gyrase and topoisomerase (Fig. 41.2). The first of these targets is DNA gyrase, a type II topoisomerase, composed of two *gyrA* subunits and two *gyrB* subunits [4]. The second target for the fluoroquinolones is topoisomerase IV, composed of two *parC* subunits and *parE* subunits, respectively. The protein subunits coded by *parC* and *parE* are homologous to the *gyrA* and *gyrB* subunits, respectively. These DNA gyrase and topoisomerase were coded by *gyrA*, *gyrB*, *parC*, and *parE* in the quinolone resistance-determining region (QRDR). Quinolone antimicrobial agent can bind to DNA gyrase complex and prevent DNA gyrase dissociation. This interrupts DNA fragmentation and eventually stops replication process.

41.3 Epidemiology of Fluoroquinolone Resistance

Fluoroquinolone resistance of *H. pylori* was not reported until 2005. Kim et al. demonstrated that ciprofloxacin, levofloxacin, and moxifloxacin's minimal inhibitory concentrations (MICs) tend to shift more higher during the period of 1987–2003 [5]. The resistance rates of fluoroquinolones were steadily increased initially from 13.9% (1994) to 33.8% (2003).

The isolates obtained from patients without previous *H. pylori* eradication treatment were defined as primary. However, for patients with previous unsuccessful eradication, the isolates were defined as secondary strains. Trends for the prevalence of fluoroquinolone resistance rates in primary and secondary clinically isolated *H. pylori* were published in 2013 [6]. Trends of MICs in *H. pylori* isolates during three periods of time (2003–2005, 2006–2008, and 2009–2012) were investigated. Increased trends regardless of the primary and secondary isolates was found in both of levofloxacin and moxifloxacin (5.7–34.6%, $p < 0.001$) during 2003–2012 [6]. It is inferred that increasing trends of fluoroquinolones might be caused by higher prescription rate at local clinics in Korea, especially otorhinolaryngological and respiratory diseases. However, this result might have selection bias by recruiting study subjects only from a referral center. In the report on the fluoroquinolone trend until 2022, resistance in Korea was still high up to 35.7% (Fig. 41.3). Meanwhile, another report demonstrates that the problems with high resistance rates of fluoroquinolone were prevalent between three hospitals with different location in Korea, all above 20% [7] (Fig. 41.4).

Increased fluoroquinolone resistance is also a worldwide problem, especially for Asian countries including Taiwan, China, and Japan, even more European countries [9] (Fig. 41.5). The study performed in Taiwan reported about 10% fluoroquinolone resistance rate in 2004. For Japan 2007, about 15% of fluoroquinolone resistance rate was reported [10]. Differences of fluoroquinolone resistance rate between countries were more enlarged, especially for European countries. It has been well known that the resistance rate of fluoroquinolone in Italy was low. However, recent published articles reported increased resistance rate up to 22.1%. The study performed in Greece over 10 years (2000–2010) demonstrates only small increase of fluoroquinolone resistance, 0–5.7% [11]. For United Kingdom, 17% of resistance rate was reported in 2012 [12]. But research performed in Portugal reported high prevalence of resistance up to 33.9% [13, 14]. This fluoroquinolone resistance in Europe was also rechecked in a follow-up

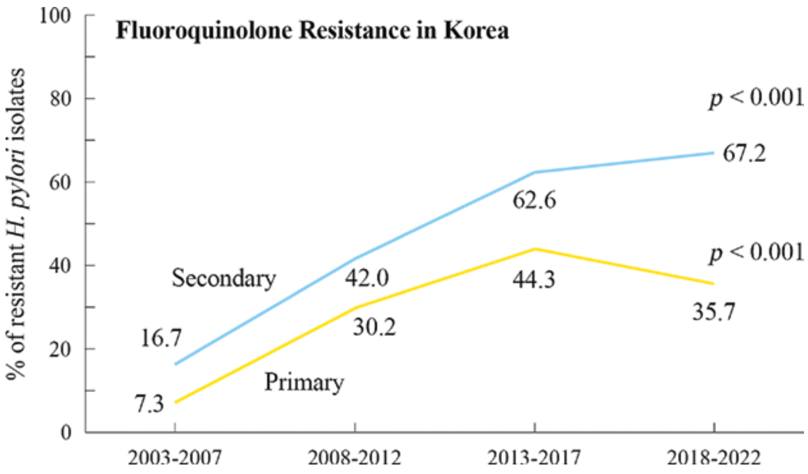

Fig. 41.3 Change of fluoroquinolone resistance rate. (Adapted from Lee et al. [6, 8])

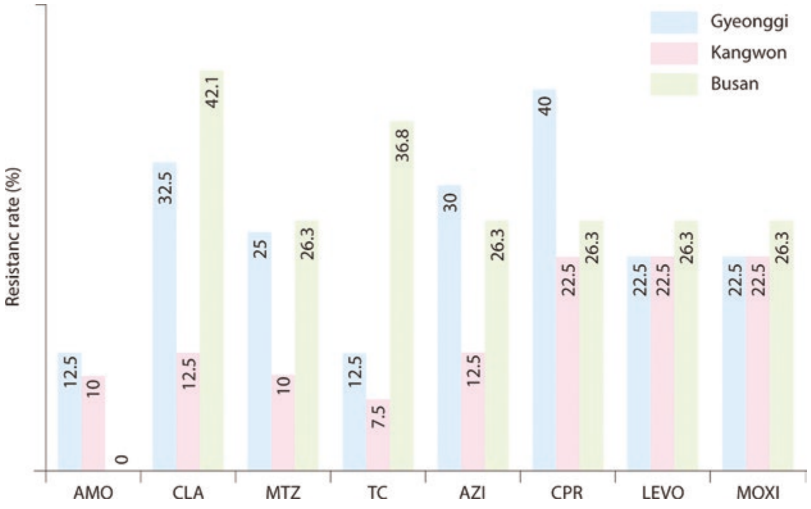

Fig. 41.4 Regional difference of antimicrobial resistance rate. *AMO* amoxicillin, *CLA* clarithromycin, *MTZ* metronidazole, *TC* tetracycline, *AZI* azithromycin, *CPR* ciprofloxacin, *LEVO* levofloxacin, *MOXI* moxifloxacin. (Adapted from Kim et al. [7])

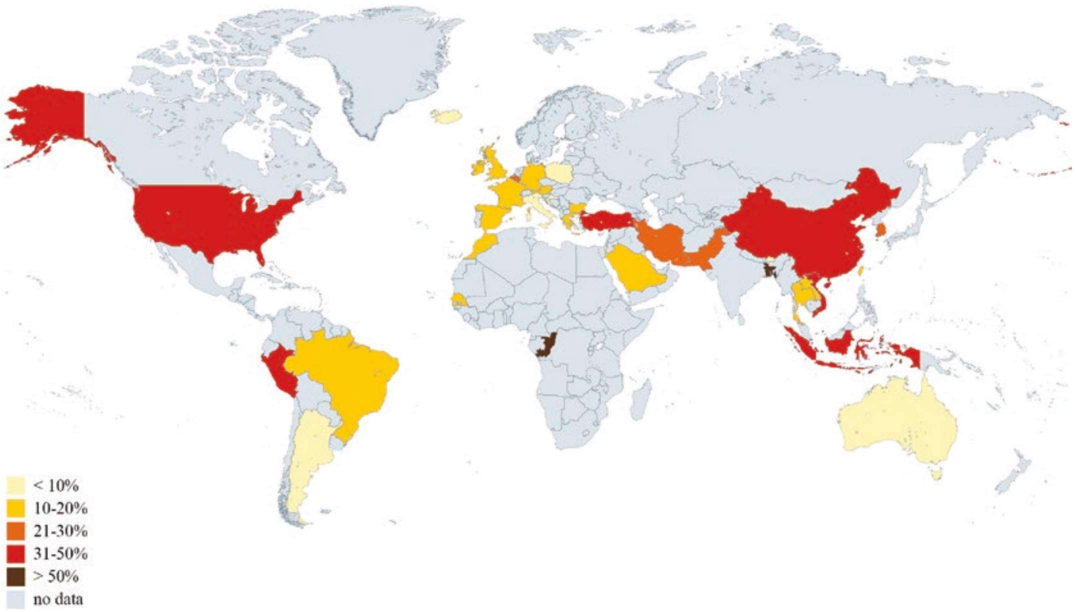

Fig. 41.5 Pooled prevalence (2006–2016) of resistance to levofloxacin. (Adapted from Savoldi et al. [9])

study in 2018, showing a high resistance rate of over 20% in Italy (29.2%), Bulgaria (27.8%), Portugal (24.4%), and Belgium (23.2%), respectively [15].

The study performed in Brazzaville, Congo, demonstrates 50% rate of resistance [16]. Consequently, the recent worldwide increase in resistance has become a significant limitation for providing effective eradication treatment using fluoroquinolone [17]. These results are in agreement with the previous studies in European countries and in Japan. Commonly, resistance rate of secondary *H. pylori* isolates tends to be higher compared with primary isolates. From this background, Megraud et al. [18] also suggested that increasing trends of fluoroquinolone resistance rate might correlate with overall fluoroquinolone consumption. The present authors also agreed this statement when considering the rapidly increasing trends reported in Korea [18].

41.4 Diagnosis of Fluoroquinolone Resistance

The most reliable diagnostic method for the determination of fluoroquinolone susceptibility is agar-dilution method. But E-test also has been widely used for their simplicity and low cost. The Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommended diagnostic procedures and cutoff values at their guidelines. However, the cutoff value of fluoroquinolone was only provided by EUCAST, MIC above 1.0 μg/mL. Hence, majority of research articles about fluoroquinolone for *H. pylori* followed breakpoints of >1.0 μg/mL as published elsewhere provisionally, not as a guideline. In my opinion, breakpoints of fluoroquinolone for *H. pylori* should be updated upon substantially accumulated evidences.

41.5 Mechanism of Fluoroquinolone Resistance

41.5.1 Mechanism of Fluoroquinolone Resistance of *H. pylori*

There are four different mechanisms of fluoroquinolone resistance discovered by the study of

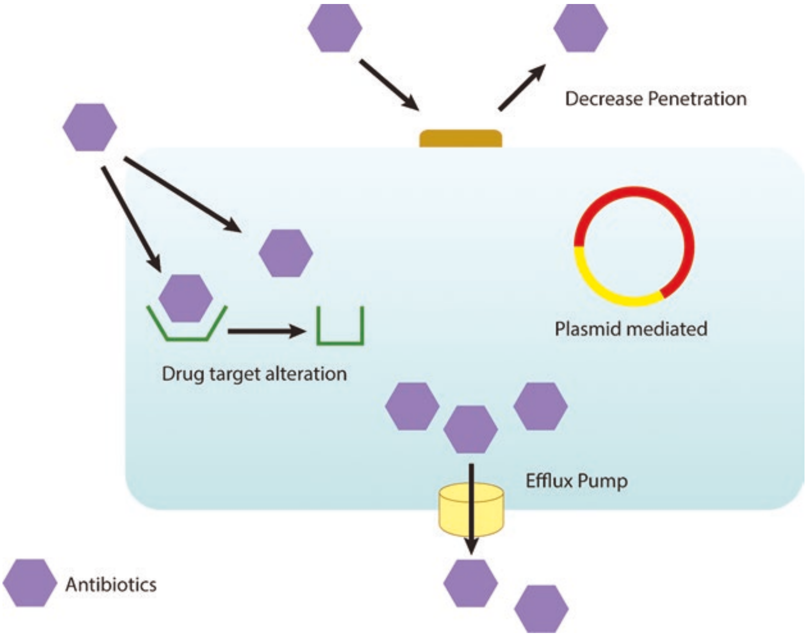

Fig. 41.6 Resistance mechanism of fluoroquinolone

other bacteria. These mechanisms include the alteration of drug targets such as DNA gyrase or topoisomerase IV, the activation of a drug efflux pump system, a decrease of drug permeability in the membrane, and the plasmid-mediated resistance (Fig. 41.6).

The most important resistance mechanism is target alteration. Fluoroquinolones usually inhibit DNA gyrase for Gram-negative and topoisomerase IV for Gram-positive bacteria. This mode of action could be achieved by binding of fluoroquinolone antibiotics to bacterial gene which encoded gyrase or topoisomerase IV. However, if the bacterial gene experiences critical point mutation which could cause three-dimensional conformational changes, binding of fluoroquinolone could not happen, and it can cause the development of resistance. In addition, active efflux mechanism of *H. pylori* could be responsible for the development of multidrug resistance. Efflux pump can eliminate intracellular toxic compound to extracellular and is known to have an important role in the development of multidrug resistance. Efflux pump of *H. pylori* was encoded for *hefA* gene of *H. pylori* genome [19]. Usually intrinsic resistance might be related to the presence of efflux pump. But acquired resistance could be developed by efflux pump overexpression. It was assumed that altered drug permeability and plasmid-mediated resistance might be present in *H. pylori*. But the impact of altered drug permeability and plasmid-mediated resistance were still not investigated. Additionally, any material which can directly inactivate antibiotics like β-lactamase was not reported in *H. pylori*.

41.5.2 Distinct Feature of Genes Responsible for Fluoroquinolone Resistance

Until present, the most effective and decisive mechanism of resistance for fluoroquinolone is the target alteration of binding site. However, *H. pylori* do not possess *parC* or *parE* genes that encode the topoisomerase IV unlike other species [20]. Therefore, mutation of DNA gyrase genes, especially *gyrA*, is known to play an important role in the determination of antimicrobial susceptibility. And it has been also suggested that *gyrB* mutations did not play an important role in fluoroquinolone resistance in *H. pylori* [21].

Therefore, this difference of genome must be the critical reason why *H. pylori* strains with single-point mutations of *gyrA* appear to be more difficult to overcome than other species of microorganisms whose inhibition of topoisomerase IV still negatively affects the growth of pathogens. In conclusion, it could be explained that fluoroquinolones have limited target to *H. pylori*, approximately half, compared to other common bacterial species.

41.6 Present Status of the Studies About Fluoroquinolone Resistance

The cornerstone research about resistance mechanism of fluoroquinolone for *H. pylori* was published in 2003 by Tankovic et al. [21]. They performed determination of MICs by the agar-dilution method for nine ciprofloxacin-susceptible and two ciprofloxacin-resistant isolates. They found that the presence of a *gyrA* mutation was associated with higher MIC values. So, they concluded that the presence of a *gyrA* mutation might have decisive role in the determination of susceptibility. And it is inferred that the impact of *gyrB* might not be decisive at least for *H. pylori*.

The first systematic report regarding fluoroquinolone resistance gene in Korea was published in 2005 by Kim et al. [5]. The authors assessed the prevalence rate of primary fluoroquinolone resistance over the 16 years (1987–2003). Moreover, authors demonstrate distribution of point mutation of the *gyrA* gene at A272G and G271A by sequence analysis in 14 fluoroquinolone-resistant strains at first in Korea.

The most notable and frequently cited report was published in 2006 by Nobuo Aoyama in Kobe, Japan [8]. The authors performed levofloxacin susceptibility test by E-test in clinically isolated *H. pylori*. Pattern of mutations in the quinolone resistance-determining regions of the *gyrA* and *gyrB* genes was evaluated by direct sequencing of 68 levofloxacin-resistant and 50 susceptible strains. There was a significant difference in the presence of *gyrA* mutations between levofloxacin-resistant (83.8%) and levofloxacin-susceptible (14.0%) strains. A notable point in this study is the analysis regarding the difference of impact which is affected by the location of *gyrA* point mutation, Asn-87 or Asp-91. So the authors concluded that *gyrA* mutations at Asp-91 tended to be associated with low-level resistance, but those at Asn-87 with high-level resistance.

In 2011, the study about the distribution of *gyrA* point mutations and overall resistance rate of fluoroquinolone in Korea was published [22]. This research performed *H. pylori* isolation from the patients. DNA sequencing of the QRDR of *gyrA* was performed in 89 fluoroquinolone-resistant and 27 fluoroquinolone-susceptible isolates. The most common mutations were Asp-91 (36.0%) and Asn-87 (33.7%). The most common substitution of *gyrA* in Asn-87 was found to be Asn-87-Lys. In the case of Asp-91, Asp-91-Asn was the most common. Additionally, the most important point of the study was the results about the outcomes of fluoroquinolone-containing treatment. Eradication failure was only noted for two cases with the Asn-87-Lys mutation. But, no treatment failure was observed among the three patients with Asp-91-Asn mutated strains. Moreover, Rimbara et al. [23] also had similar opinion. They performed a series of experiments consisted of MIC, sequencing, and transformation. The authors proposed that mutation from Asn-87-Lys at *gyrA* confers higher resistance to levofloxacin and gatifloxacin compared with the mutation dose from Asp-91-Asn. In conclusion, Asn-87 point mutation might be a more important determinant for failure of fluoroquinolone-containing eradication compared with Asp-91 mutation.

41.7 Perspective and Limitation of Studies About Fluoroquinolone Resistance Mechanism

The studies about the mechanism of fluoroquinolone resistance could be applicable for two aspects. First, it could be the basis for the development of diagnostic tool for antimicrobial resistance. Second, it could be helpful to find appropriate therapeutic target for overcoming resistance.

As described earlier, the major mechanism of fluoroquinolone resistance, especially for *H. pylori*, is the point mutation of the Asn-87, Asp-91 of *gyrA*. This inspired the development of the molecular diagnostic method that combines polymerase chain reaction (PCR) and DNA strip hybridization. GenoType® HelicoDR (Hain Lifescience, Germany) could be directly applicable to gastric biopsy specimens and could draw result within 6 h. GenoType® HelicoDR test could identify majority of point mutation of 23S rRNA and *gyrA*.

Researches about the evaluation of clinical usefulness of GenoType® HelicoDR test were performed and published in 2014 in Korea [24]. The sensitivity and specificity of GenoType® HelicoDR test in determination of 23S rRNA mutation were 94.9% and 87.1%, and those of *gyrA* 98.2% and 80.0%, respectively. But this research could not be extended because of the different prevalence of fluoroquinolone resistance compared with those of other countries. Relatively low sensitivity and false-positive rate were additional problems. But with the presence of several shortcomings, it showed high negative predictive value for detecting 23S rRNA (96.4%) or *gyrA* (97.4%) mutations. It is inferred that GenoType® HelicoDR test could be effectively used in predicting treatment failure due to resistance of clarithromycin or fluoroquinolone. Additionally, dual-priming oligonucleotide (DPO)-based multiplex PCR method used for the detection of 23S rRNA mutation (clarithromycin resistance) applied for *gyrA* mutation [25]. However, clinical trial results using PCR method have not been reported. Recently, resistance determination through genomic analysis using the next-generation sequencing method showed favorable results [26, 27].

Novel fluoroquinolones such as sitafloxacin, gemifloxacin, and finafloxacin have been developed and studied [28–30]. Studies about these novel fluoroquinolones generally demonstrated positive favorable outcomes. These fluoroquinolones commonly presented the proofs that these materials even could overcome resistance caused by *gyrA* mutations. However, these novel fluoroquinolones should undergo sufficient preclinical stage and should find suitable indications. Moreover, the most fundamental unsolved problems about resistance mechanism were the presence of resistance caused by mutations other than *gyrA* mutations. Nevertheless, it is reasonably explained that mutations of 23S rRNA were present at clarithromycin resistance *H. pylori* > 90% [31]. But for fluoroquinolone, only 70–80% of resistance strains have the point mutation of *gyrA* [8, 22, 23]. Above all things, the authors concluded that further efforts are needed to elucidate the complete mechanism of fluoroquinolone resistance.

Megraud et al. [18] recommended in 2013 that fluoroquinolone antibiotics should be prescribed only for patients without prior history of fluoroquinolone treatment for any cause. Moreover, in the latest European guideline published in 2022, it was still recommended to reserve fluoroquinolone-containing therapy due to problems of resistance [32]. Considering the updated results of fluoroquinolone antibiotics, fluoroquinolones have sufficient efficacy and bioavailability even with a few side effects. These antibiotics deserve further dedication of efforts.

41.8 Conclusions

Fluoroquinolone antibiotics show excellent antimicrobial efficacy to *H. pylori* with fewer side effects. However, the recent increase of fluoroquinolone resistance has become a significant limitation for providing effective eradication. Unlike common bacteria, *H. pylori* do not have the *parC* or *parE* genes which encode topoisomerase IV. Therefore, mutation of DNA gyrase genes, especially Asn-87 point mutation of *gyrA*, is known to play a decisive role in the determination of susceptibility. Epidemiologic study for mutations of resistance gene might be helpful for the development of molecular diagnostic tool and novel fluoroquinolone agent. In conclusion, further efforts are needed especially to devise new therapeutic agent, diagnostic tool, and more effective fluoroquinolone-containing regimen.

References

1. Kim SG, Jung HK, Lee HL, Jang JY, Lee H, Kim CG, et al. Guidelines for the diagnosis and treatment of *Helicobacter pylori* infection in Korea, 2013 revised edition. J Gastroenterol Hepatol. 2014;29:1371–86.
2. O'Connor A, Vaira D, Gisbert JP, O'Morain C. Treatment of *Helicobacter pylori* infection 2014. Helicobacter. 2014;19(Suppl 1):38–45.
3. Liang CM, Cheng JW, Kuo CM, Chang KC, Wu KL, Tai WC, et al. Levofloxacin-containing second-line anti-*Helicobacter pylori* eradication in Taiwanese real-world practice. Biom J. 2014;37:326–30.
4. Zhanel GG, Walkty A, Vercaigne L, Karlowsky JA, Embil J, Gin AS, et al. The new fluoroquinolones: a critical review. Can J Infect Dis. 1999;10:207–38.
5. Kim JM, Kim JS, Kim N, Jung HC, Song IS. Distribution of fluoroquinolone MICs in *Helicobacter pylori* strains from Korean patients. J Antimicrob Chemother. 2005;56:965–7.
6. Lee JW, Kim N, Kim JM, Nam RH, Chang H, Kim JY, et al. Prevalence of primary and secondary antimicrobial resistance of *Helicobacter pylori* in Korea from 2003 through 2012. Helicobacter. 2013;18:206–14.
7. Kim JY, Kim NY, Kim SJ, Baik GH, Kim GH, Kim JM, et al. Regional difference of antibiotic resistance of *Helicobacter pylori* strains in Korea. Korean J Gastroenterol. 2011;57:221–9.
8. Lee JW, Kim N, Choi SI, Jang JY, Song CH, Nam RH, et al. Prevalence and trends of multiple antimicrobial resistance of *Helicobacter pylori* in one tertiary hospital for 20 years in Korea. Helicobacter. 2023;28:e12939.
9. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of antibiotic resistance in Helicobacter pylori: a systematic review and meta-analysis in World Health Organization regions. Gastroenterology. 2018;155:1372–82.
10. Miyachi H, Miki I, Aoyama N, Shirasaka D, Matsumoto Y, Toyoda M, et al. Primary levofloxacin resistance and gyrA/B mutations among *Helicobacter pylori* in Japan. Helicobacter. 2006;11:243–9.
11. Karamanolis GP, Daikos GL, Xouris D, Goukos D, Delladetsima I, Ladas SD. The evolution of *Helicobacter pylori* antibiotics resistance over 10 years in Greece. Digestion. 2014;90:229–31.
12. McNulty CA, Lasseter G, Shaw I, Nichols T, D'Arcy S, Lawson AJ, et al. Is *Helicobacter pylori* antibiotic resistance surveillance needed and how can it be delivered? Aliment Pharmacol Ther. 2012;35:1221–30.
13. Almeida N, Romaozinho JM, Donato MM, Luxo C, Cardoso O, Cipriano MA, et al. *Helicobacter pylori* antimicrobial resistance rates in the central region of Portugal. Clin Microbiol Infect. 2014;20:1127–33.
14. Wu W, Yang Y, Sun G. Recent insights into antibiotic resistance in *Helicobacter pylori* eradication. Gastroenterol Res Pract. 2012;2012:723183.
15. Megraud F, Bruyndonckx R, Coenen S, Wittkop L, Huang TD, Hoebeke M, et al. *Helicobacter pylori* resistance to antibiotics in Europe in 2018 and its relationship to antibiotic consumption in the community. Gut. 2021;70:1815–22.
16. Ontsira Ngoyi EN, Atipo Ibara BI, Moyen R, Ahoui Apendi PC, Ibara JR, Obengui O, et al. Molecular detection of *Helicobacter pylori* and its antimicrobial resistance in Brazzaville Congo. Helicobacter. 2015;20:316–20.
17. Tai WC, Lee CH, Chiou SS, Kuo CM, Kuo CH, Liang CM, et al. The clinical and bacteriological factors for optimal levofloxacin-containing triple therapy in second-line *Helicobacter pylori* eradication. PLoS One. 2014;9:e105822.
18. Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, et al. *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut. 2013;62:34–42.
19. Zhang Z, Liu ZQ, Zheng PY, Tang FA, Yang PC. Influence of efflux pump inhibitors on the multidrug resistance of *Helicobacter pylori*. World J Gastroenterol. 2010;16:1279–84.
20. Alm RA, Ling LS, Moir DT, King BL, Brown ED, Doig PC, et al. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature. 1999;397:176–80.
21. Tankovic J, Lascols C, Sculo Q, Petit JC, Soussy CJ. Single and double mutations in *gyrA* but not in *gyrB* are associated with low- and high-level fluoroquinolone resistance in *Helicobacter pylori*. Antimicrob Agents Chemother. 2003;47:3942–4.
22. Lee JW, Kim N, Nam RH, Park JH, Kim JM, Jung HC, et al. Mutations of *Helicobacter pylori* associated with fluoroquinolone resistance in Korea. Helicobacter. 2011;16:301–10.
23. Rimbara E, Noguchi N, Kawai T, Sasatsu M. Fluoroquinolone resistance in *Helicobacter pylori*: role of mutations at position 87 and 91 of GyrA on the level of resistance and identification of a resistance conferring mutation in *GyrB*. Helicobacter. 2012;17:36–42.
24. Lee JW, Kim N, Nam RH, Park JH, Choi YJ, Kim JM, et al. Genotype HelicoDR test in the determination of antimicrobial resistance of *Helicobacter pylori* in Korea. Scand J Gastroenterol. 2014;49:1058–67.
25. Haumaier F, Schneider-Fuchs A, Backert S, Vieth M, Sterlacci W, Wöhrl B, et al. Rapid detection of quinolone resistance mutations in *gyrA* of *Helicobacter pylori* by real-time PCR. Pathogens. 2022;11:59.

26. Egli K, Wagner K, Keller P, Risch L, Risch M, Bodmer T. Comparison of the diagnostic performance of qPCR, sanger sequencing, and whole-genome sequencing in determining clarithromycin and levofloxacin resistance in *Helicobacter pylori*. Front Cell Infect Microbiol. 2020;17(10):596371.
27. Moss SF, Dang LP, Chua D, Sobrado J, Zhou Y, Graham DY. Comparable results of *Helicobacter pylori* antibiotic resistance testing of stools vs gastric biopsies using next-generation sequencing. Gastroenterology. 2022;162:2095–7.
28. Sugimoto M, Sahara S, Ichikawa H, Kagami T, Uotani T, Furuta T. High *Helicobacter pylori* cure rate with sitafloxacin-based triple therapy. Aliment Pharmacol Ther. 2015;42:477–83.
29. Chang WL, Kao CY, Wu CT, Huang AH, Wu JJ, Yang HB, et al. Gemifloxacin can partially overcome quinolone resistance of *H. Pylori* with *gyrA* mutation in Taiwan. Helicobacter. 2012;17:210–5.
30. Lee JW, Kim N, Nam RH, Kim JM, Park JY, Lee SM, et al. High antimicrobial efficacy of finafloxacin on *Helicobacter pylori* isolates at pH 5.0 compared with those of other fluoroquinolones. Antimicrob Agents Chemother. 2015;59:7629–36.
31. Hwang TJ, Kim N, Kim HB, Lee BH, Nam RH, Park JH, et al. Change in antibiotic resistance of *Helicobacter pylori* strains and the effect of A2143G point mutation of 23S rRNA on the eradication of *H. Pylori* in a single center of Korea. J Clin Gastroenterol. 2010;44:536–43.
32. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.

Metronidazole

42

Sun Min Lee

Abstract

Metronidazole is one of the major antibiotics for the eradication of *Helicobacter pylori* (*H. pylori*). The metronidazole resistance rate of *H. pylori* is relatively high compared to other antibiotics. It appears to be different among nations and areas with a tendency to increase. Gene mutations in the genes encoding nitroreductases have been majorly studied as the mechanism of metronidazole resistance. Representative genes related to metronidazole resistance are *rdxA* and *frxA*. The gene mutation patterns appear differently in *H. pylori* strains isolated from patients. The effect of the gene mutations is proven by confirming the induction of resistance with transformation of mutated *rdxA*. Efflux pump system is also involved in the occurrence of resistance. In resistant strains, *hefA*, the gene encoding the efflux pump, is highly expressed, and this status results in the inhibition of drug influx. Overall, *hefA* is overexpressed in the early stage of the resistance to inhibit the influx of the medicine; then the mutations in *rdxA* and *frxA* cause the abnormal activity of nitroreductases resulting in reinforced resistance.

Keywords

Antibiotic resistance · *Helicobacter pylori*
Metronidazole

42.1 Introduction

Metronidazole, a nitroheterocyclic compound, is one of the major drugs used for the eradication of *Helicobacter pylori* [1]. This antibiotic is effective in diverse bacterial or protozoal infections including *H. pylori* infections, pelvic inflammatory disease, endocarditis, bacterial vaginosis, dracunculiasis, giardiasis, trichomoniasis, and amebiasis. It is activated through reduction by anaerobic microorganisms, so it is majorly used for eradication of anaerobic bacteria or protozoa. The resistance rate of metronidazole which has increased worldwide is higher than that of other drugs and affects the eradication outcome [2–5]. Therefore, it is important to prevent the occurrence of resistance and provide solutions for the resistant bacteria by understanding the mechanisms.

In this chapter, we will discuss the mechanism of action, the present conditions, and mechanisms of metronidazole resistance.

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42.2 Epidemiological Aspects of Metronidazole Resistance

42.2.1 The Resistance Rate

Metronidazole-resistant *H. pylori* are found worldwide, and the resistance rate differs according to nations. USA regions have shown resistance rate of 25.1% in 1998–2002 [6] and 43.6% in 2017–2018 [7]. The resistance rate in Northern, Central/Western, and Southern Europe was respectively 28.6%, 43.8%, and 29.7% in 2008–2009 [8]. Then it was investigated to be 38.9% in Europe overall in 2018 [9]. Some areas in Asia and Middle East have relatively high resistance rates [10–14] (Table 42.1). The metronidazole resistance rate of *H. pylori* is also different in several regions of South Korea. As investigated in 2008, the resistance rate was the lowest at Gangwon (10%, *n* = 40), compared with Gyeonggi (25%, *n* = 40) and Busan (19.2%, *n* = 19) (Table 42.2). Two hospitals in Seoul were shown to have high resistance rate of 50–66%, according to research in 1989, 1994, and 2003 [3]. Recently, there was the first nationwide study in South Korea that investigated antibiotic resistance of *H. pylori* over the geographic area of South Korea during 2017–2018 [17]. They collected samples from 15 clinical centers of six geographic regions and tested the antibiotic resistance in a single center. Among 590 enrolled subjects, they could obtain the antibiotic resistance data of 349 samples. Conclusively, they reported that the overall metronidazole resistance rate was 29.5% in South Korea, and that the tendency of antibiotic resistance differed by antibiotic use and geographic area, insisting the necessity for effective eradication strategies.

Studying the changing aspect by year, the resistance rates appeared to increase or persist. The tendency was shown in several hospitals in South Korea; at Hanyang University Hospital, it increased from 52.9% to 66.2% as investigated in 1987 and 2003, and at Samsung Medical Center, it was 33.3% in 1994 and 47.7% in 1998–1999 [5]. At Seoul National University Bundang Hospital which is located in Gyeonggi province, it was 34.8% in 2003–2005, 23.8% in 2006–2008, and 35.8% in 2009–2013 [18].

42.2.2 Correlations Between the Resistance and the Eradication Failures

The *H. pylori* eradication rates tend to decrease with high resistance rates [19]. The gap appeared differently according to the eradication therapy; eradication rate declined from 93% to 19%, and it retained 94% in some cases [20, 21]. The difference of eradication rates between resistant strains and susceptible ones was analyzed in 1996–1997, and regimens with eradication rates higher than 90% were BAM, BTeM, OAM, LCM, OCM, and BTML (A, amoxicillin; B, bismuth citrate; C, clarithromycin; L, lansoprazole; M, metronidazole; O, omeprazole; Te, tetracycline) (Table 42.3). Among them, the eradication

Table 42.1 Metronidazole resistance rates of *H. pylori* according to nations (%)

Regions	USA		Europe				Middle East	Asia			
			Northern [8]	Central/western [8]	Southern [8]	Overall [9]	Israel [10]	Kolkata (India) [11]	Beijing (China) [12]	Japan [13]	Malaysia [14]
Year	1998–2002 [6]	2017–2018 [7]	2008–2009	2008–2009	2008–2009	2018	2000–2001	2000–2001	2009–2010	2002–2005	2017–2021
Resistance rate (%)	25.1	43.6	28.6	43.8	29.7	38.9	60.7[a], 38.2[b]	85	61.6	3.3–5.3	82.4

Adapted and updated from Lee and Kim [15]

Studied nations, patient enrolment periods, and metronidazole resistance rate (%) (metronidazole-resistant if MIC ≥8 μg/mL)

[a] Resistance rate in patients treated with metronidazole

[b] Resistance rate in patients not treated with metronidazole

Table 42.2 Metronidazole resistance rates of *H. pylori* in South Korea (%)

Regions	Seoul [5]					Seoul [3]			Gyeonggi [16]	Gangwon [16]	Busan [16]	Overall [17]
Year	1994	1995	1996	1997	1998–1999	1987	1994	2003	2008	2008	2008	2017–2018
Resistance rate (%)	33.3	38.5	42.6	40.6	47.7	52.9	61.1	66.2	25.0	10.0	19.2	29.5

Adapted and updated from Lee and Kim [16]
Resistance rate (%) means the rate of patients with resistant *H. pylori* in total enrolled patients

Table 42.3 Difference of eradication rates according to metronidazole resistance

Treatment	Reference	Metronidazole-susceptible	Metronidazole-resistant
BAM	Logan et al. [22]	93	19
	Burette et al. [23]	90	63
	Rautelin et al. [24]	91	63
BTeM	Noach et al. [21]	96	38
	Lerang et al. [20]	92	79
OAM	Lerang et al. [25]	96	77
	Thijs et al. [26]	95	69
LCM	Harris et al. [27]	92	75
OCM	Lerang et al. [25]	94	94
OCTi	Moayyedi et al. [28]	70	30
BM	DeCross et al. [29]	81	16
BTMO	Van der Hulst et al. [30]	81	82
BTML	Graham et al. [31]	100	41

Adapted from Alarcón et al. [19]
Eradication rate (%) of *H. pylori* isolated from patients
A amoxicillin; *B* bismuth subcitrate; *C* clarithromycin; *L* lansoprazole; *M* metronidazole; *O* omeprazole; *Ti* tinidazole; *Te* tetracycline

rate of resistant strains with the triple therapy OCM was the same with susceptible strains (94% vs. 94%) [25]. Also in the case of BTMO, there was no significant difference between susceptible and resistant strains: 81% and 82% each [31]. This indicates that metronidazole resistance would be overcome in some cases [20, 24, 26], whereas with BAM or BTeM, the eradication rate fell from 90–96% to 19–79% [20–24]. Also, there were great differences in the cases treated with BM (81% vs. 16%) and BTML (100% vs. 41%) [32, 33]. Moreover, a lower eradication rate is associated with sequential treatment which is being suggested as an alternative regimen of the standard triple therapy [34].

The relation with metronidazole resistance and *H. pylori* eradication rate was also confirmed in animal studies. ICR mice were infected with either metronidazole-resistant or metronidazole-susceptible *H. pylori* strains. As a result, the eradication rate was significantly lower in the mice infected with resistant bacteria (Table 42.4). Therefore, the correlation between resistance and eradication failure was confirmed in vivo. However, in this case, only metronidazole was used, not applying the triple therapy or other combinations, so it cannot be considered as the practical therapy conditions. The significant difference in eradication rates according to resistance can be determined with further animal studies with other therapies.

Table 42.4 Change of eradication rate after induction of metronidazole resistance in ICR model

	Eradication rate (%)[a]	
Metronidazole (mg/kg)	Metronidazole-susceptible	Metronidazole-resistant
100	5/5 (100)	3/5 (60)
32	4/5 (80)	0/5 (0)
10	2/5 (40)	0/5 (0)

Adapted from Matsumoto et al. [32]

Eradication rate (%) of treatment with metronidazole (10, 32, 100 mg/kg) in ICR mice infected with metronidazole-susceptible or metronidazole-resistant *H. pylori*

[a] Eradication rate (%) = (number of mice eradicated)/(total number of mice) × 100

42.3 Principles of Metronidazole Resistance

42.3.1 Mode of Action

Metronidazole is one of the 5-nitroimidazole drugs which are activated as antibiotic metabolites through intracellular reduction. In the reduction process, the nitro group (RNO_2) is reduced to anionic radical ($RNO_2^{\bullet -}$), RNO, and RNHOH (chemical formula 42.1):

$$RNO_2 \rightarrow RNO_2^{\cdot -} \rightarrow RNO \rightarrow RNHOH \tag{42.1}$$

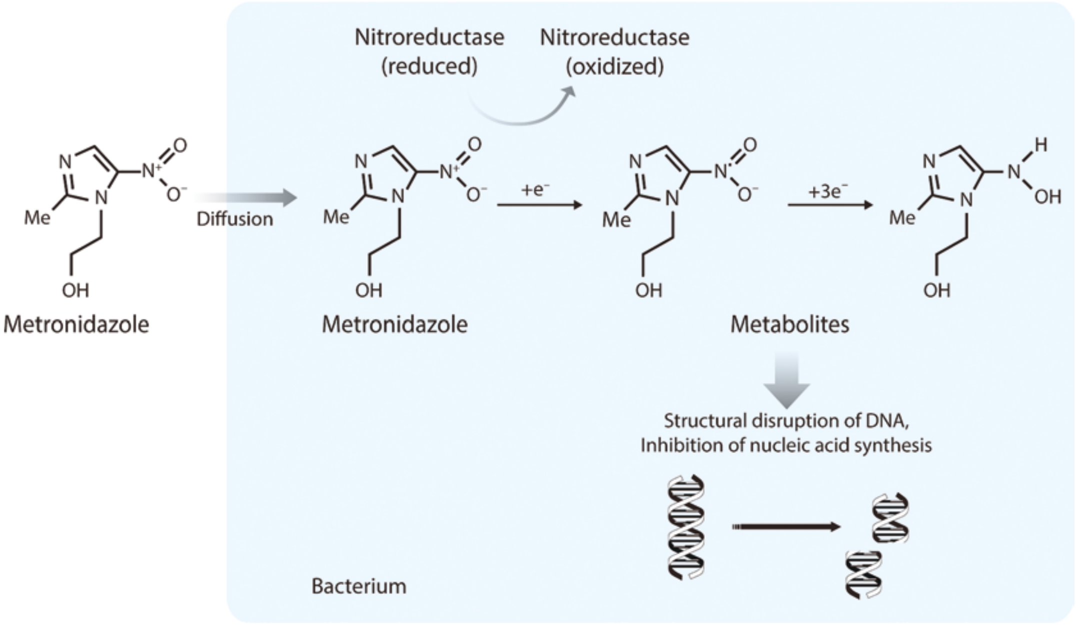
Nitroreductase (reduced)
Nitroreductase (oxidized)
N
N
Me
OH
N+
O
O−
Diffusion
Metronidazole
Metronidazole
+e−
+3e−
H
OH
Metabolites
Structural disruption of DNA, Inhibition of nucleic acid synthesis
Bacterium

Fig. 42.1 Mode of action of metronidazole. (Modified from Mann [37])

The metabolites disrupt the structure of DNA, by diminishing the helical structure and breaking the strands, and cause apoptosis by inhibiting nucleic acid synthesis [35–37] (Fig. 42.1). Because metronidazole is in low redox potential (−486 mV), it is only reduced in cells with electron donor with lower redox. This makes the medication specifically toxic in anaerobic bacteria. Metronidazole is diffused through cellular membranes, and the inflow is facilitated by decrease of intracellular concentration by the metabolic reactions.

42.3.2 Mechanism of Resistance

The most reliable hypothesis for the resistance mechanism is the obstruction in the reduction process. In this hypothesis, electron transport proteins in *H. pylori* are not expressed because of the mutations encoding the proteins. The reductases are ferredoxin (FdxA), flavodoxin (FldA), ferredoxin-like proteins (FdxB, OorDABC, PorCDAB), NAD(P)H flavin oxidoreductase (FrxA), and oxygen-insensitive NAD(P)H nitroreductase (RdxA). Particularly, *rdxA* and *frxA*, genes encoding RdxA and FrxA, have been considered as causative factors of resistance. As described above, many researches have been performed to understand the mechanism of metronidazole resistance. In this section, studies up to date would be discussed.

42.3.2.1 Mutations in *rdxA* and *frxA*

The pattern of point mutations in *rdxA* appears different in each metronidazole-resistant strain. Thirty strains isolated from patients were compared to *rdxA* sequences, and 122 different point mutations were found [38]. Missense mutations (frameshifts, nucleotide substitution), nonsense mutations (change to stop codon) that prevent normal protein expression, and nucleotide insertions that prevent normal protein expression were all observed. *rdxA* mutations comprised amino acid substitutions compared with other housekeeping genes because *rdxA* frequently has mutations in the first two codons, not the third codon (the wobble position), which affects the amino acid identity [38, 39]. Another study analyzed the upstream and whole sequences of *rdxA* and *frxA* in 19 isolated strains comparing according to the metronidazole resistance, to find the random pattern of point mutations. Resistant strains had nonsense mutations or missense mutations. The gene mutations were shown to be different in all strains, and two types of mutations were commonly found in two isolated strains: G to A at position 401 of *rdxA* and G to T at position 624 of *frxA* [39] (Table 42.5). In summary, gene mutations of *rdxA* and *frxA* occur distinctly in different strains and certain genotypes that induce metronidazole resistance are not determined at present.

On the other hand, in terms of the *rdxA* mutation types, one study provided an aspect that some RdxA amino acid changes may be signals for phylogenetic changes rather than the antibiotic resistance [40]. The research group investigated on the RdxA amino acid changes in 238 clinical strains from Shenzhen, China (where the *H. pylori* infection was endemic). Among the RdxA mutations, S88P had been reported as mutations found only in metronidazole-resistant *H. pylori* strains. The study group suggested that S88P might not be the causative factor for metronidazole resistance by showing that S88P was a conserved mutation found only in the Shenzhen strains, not in the *H. pylori* strains used for previous phylogenetic studies (strains obtained from Aboriginal Australians or from those who moved to Australia during the past 200 years) [40, 41]. Therefore, the possibility of phylogenetic changes should be considered before defining the variants as resistance factors.

To prove that *rdxA* and *frxA* mutations cause the metronidazole resistance, it should be demonstrated that the resistance occurs by the *rdxA* and *frxA* variants. Two independent studies confirmed that the metronidazole-susceptible *H. pylori* acquire resistance through transformation experiments [42, 43]. The results proved the role of *rdxA* mutations in the antibiotic resistance. Metronidazole-resistant strains were rendered susceptible by transformation with a functional *rdxA* gene; conversely, susceptible strains were rendered resistant by *rdxA* inactivation [42].

Table 42.5 Mutations of *rdxA* and *frxA* genes and RdxA and FrxA proteins in metronidazole-resistant *H. pylori*

		Mutation			
H. pylori strain	MIC (μg/mL)	*rdxA* gene mutation[a]	RdxA amino acid mutation[c]	*frxA* gene mutation[a]	FrxA amino acid mutation[c]
0844	8	G169T	V57F	A104C	E35A
1247	8	G184A	V62I	G624T	K208N
0937	12	G56A	C19Y	G391T	E131stop
0888	16	G223T	E75stop	C394T	E131stop
0871	32	N[b]	N[b]	TT160-1 insertion; A163T	L54F; K55stop
1021	48	G103T	E35stop	T540A	Y180stop
1221	128	C148T	Q50stop	G482T	C161F
				G624T	K208N
1202		T162 deletion	F54L	N[b]	N[b]
		G163T	V55 stop		
1122	128	G401A	S134N	A54 deletion	K18N
				T116A	L39stop
1199	256	A187T	K63stop	T271 deletion	Y91M
				G295T	V99stop
0940	>256	G401A	S134N	C215T	L71F
				A217G	S72G

Adapted from Han et al. [39]

RdxA oxygen-insensitive NAD(P)H nitroreductase; *FrxA* NAD(P)H flavin oxidoreductase; *MIC* minimum inhibitory concentration

[a] Nucleotide A from the first codon (ATG) for FrxA was counted as +1

[b] No specific changes as compared with genes or proteins from metronidazole-susceptible *H. pylori* isolates

[c] The initiation amino acid (methionine) was counted as +1

Expression in *Escherichia coli* of *frxA* made this naturally resistant species metronidazole-susceptible, and *frxA* inactivation reinforced metronidazole resistance in cells with no *rdxA,* but had little impact on the metronidazole susceptibility of *rdxA*+ bacteria [43]. Overall, it could be concluded that some *rdxA* mutations induce metronidazole resistance, and *frxA* mutations enhance the resistance.

42.3.2.2 Structural Alterations in RdxA

RdxA is composed of two functional homodimers and the RdxA dimer contains two flavo mononucleotide (FMN) cofactors. Each monomer is comprised of 210 amino acids that form eight α-helices and five β-strands [44] (Fig. 42.2). Martínez-Júlvez et al. analyzed the protein structure of RdxA and classified them into five types.

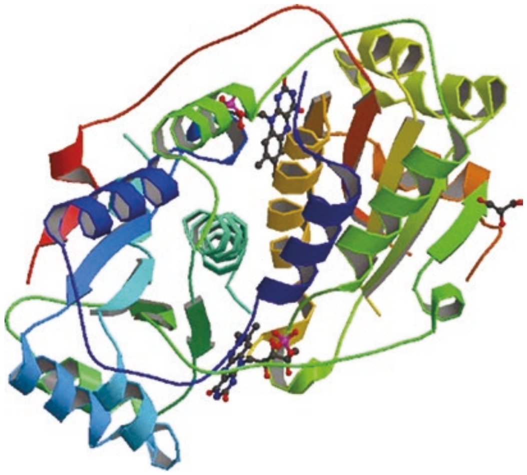

Fig. 42.2 Structure of *H. pylori* RdxA (Image from the RCSB PDB (www.rcsb.org) of PDB ID 3QDL. Adapted from Martínez-Júlvez et al. [44] and Berman et al. [45]). RCSB PDB is a member of worldwide PDB (wwPDB) (www.wwpdb.org). (Adapted from Berman et al. [46])

The first one is the mutation type that decreases the affinity of apoprotein to FMN cofactors. RdxA normally combines with FMN cofactors by hydrogen bonding, static electricity, and hydrophobic interactions; however, mutations in the side chain that combine to FMN may cause weak bonding of RdxA and FMN. This type includes mutations in R16, S18, K20, N73, I142, G162, and K200. The second type is associated with the instability of the RdxA dimer structure. Mutations in the positions of dimer boundaries L42, S43, R41, Q50, V55, M56, I142, G145, K202, and L209 are expected to induce the structural instability. The third type indicates the mutations in the positions around the redox center of FMN. These are suggested to affect the redox function of the enzyme through induction of certain chemical characteristics. Mutations in C19, Y47, and C159 can be included in this type. The fourth type including mutations in G149 and H17 destabilize other protein structures but dimer structures. Lastly, mutations that occurred in A143 and V192 affect the resistance but the mechanism cannot be explained currently. These understandings help us to understand how *rdxA* mutations affect the structure of RdxA and induce the antibiotic resistance [44].

On the other hand, the process of metronidazole reduction can be expected analyzing structure of RdxA. Distinguishing point of RdxA structure is the cysteine residue; RdxA have six cysteine residues in one subunit, while other nitroreductases contain one cysteine residue in average. These cysteine residues are supposed to be associated with the enzyme-substrate binding. In RdxA, there is a gap called isoalloxazine ring between the Y47 and isoalloxazine of FMN, which is adequate for the enzyme-substrate binding. C184 and C159 locate near the isoalloxazine ring; however, because only C159 targets the isoalloxazine ring, C159 alone is expected to affect the enzyme reaction. It can be predicted that C159 takes part in the reduction process of metronidazole by providing a proton to the N1 atom of FMN, resulting in the decrease of redox potential of FMN [44] (Fig. 42.3).

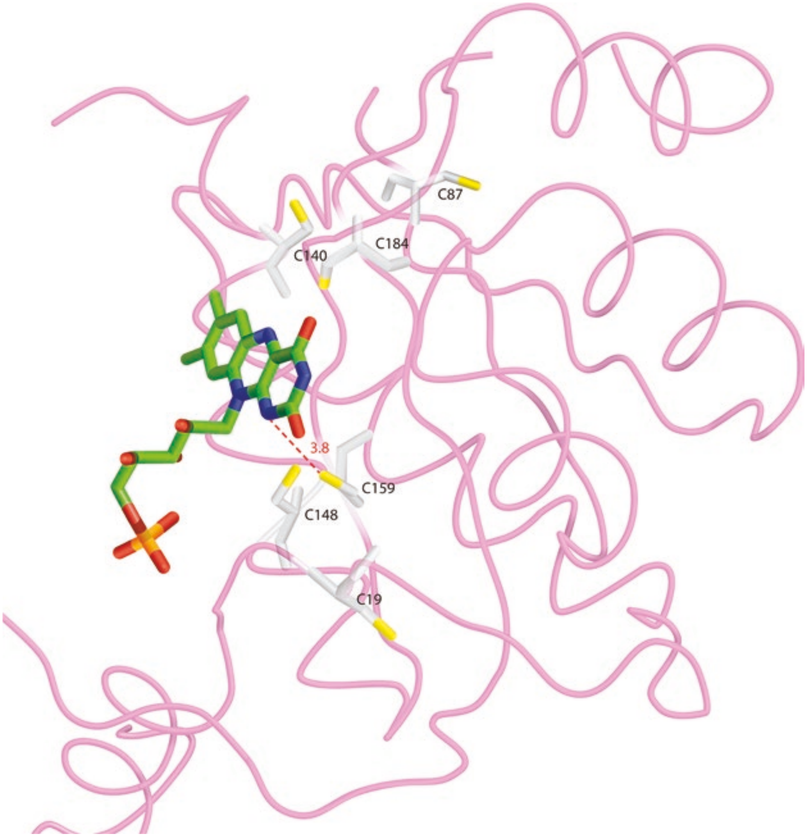

Fig. 42.3 Relative positions of the FMN and six cysteine residues in the RdxA monomer. The cysteine residues (S atoms in *yellow*) and FMN cofactor are described with a stick model. The short distance (*red dotted line*) between the C159 and the isoalloxazine ring is also shown. (Modified from Martínez-Júlvez et al. [44])

42.3.2.3 Other Mechanisms of Resistance

As discussed above, gene mutations have been suggested as the major mechanism of metronidazole resistance. However, possibilities of other mechanisms have emerged since resistance strains without specific gene mutations were reported. In the experimental results regarding this issue, 11 among 15 metronidazole-resistant-isolated strains had identical *rdxA* sequence with susceptible ones [47]. Another study reported that three among ten isolated strains were found to have metronidazole resistance although they were determined as *rdxA*+*frxA*+ by real-time PCR [48]. These results prove the existence of other mechanisms.

A novel mechanism is the efflux of metronidazole through overexpression of *hefA*, the gene encoding TolC efflux pump. In a research, the transcriptional level of RND efflux pump system was investigated in the early stage of resistance [49]. *H. pylori* were induced to acquire metronidazole resistance through exposure to metronidazole with lower concentration than the minimum inhibitory concentration (MIC). As a result, in the resistant strains, the expression level of TolC efflux pump increased, while there was no significant change in the reduction level of metronidazole. Furthermore, another study showed that *hefA* knockout in clinical resistant strains using plasmids caused the loss of metronidazole resistance; and *hefA* complementation reversely recovered the resistance in those strains [50]. Therefore, these studies provide evidences that *hefA* contributes to the metronidazole resistance in *H. pylori*. In addition to the *hefA*-related mechanisms, some variations other than the *rdxA* or *frxA* mutations were suggested as a potential mechanism of metronidazole resistance in *H. pylori* [51]. The authors analyzed on the whole genomes of 121 *H. pylori* clinical strains and found new candidate variations in core protein clusters (the inner-membrane protein RclC, a biotin carboxylase protein, and a putative threonylcarbamoyl-AMP synthase). Therefore, the stimulation of efflux and the changes in core protein clusters can be the unconventional mechanisms of metronidazole resistance in *H. pylori*.

42.4 Conclusions

The metronidazole resistance rate of *H. pylori* is increasing, and sometimes it affects the failure of eradication; therefore, it is necessary to control the resistance. Compliance of appropriate treatment is critical to control the resistance because the bacteria acquire metronidazole resistance when they are consistently exposed to the low concentration of the antibiotic.

Through many experimental reports, the overall mechanism of metronidazole resistance can be suggested. At early stage of resistance, overexpression of *hefA* inhibits the influx of metronidazole and then mutations in *rdxA* and *frxA* intensify the resistance. Mutations in *rdxA* and *frxA* are the major factors in the acquisition of metronidazole resistance, so there have been relatively many studies about them. The pattern of *rdxA* mutation appears to be diverse in each resistant strain. Structural analysis suggests that transition of C159 to alanine or serine is one of the important mutations that trigger structural changes. Besides the inactivation of RdxA and FrxA through gene mutations, there have been advances in finding the unknown mechanisms of metronidazole resistance in *H. pylori*. However, the comprehensive understanding of the whole mechanisms will be necessary for strategies to suppress and overcome the metronidazole resistance.

References

1. Nishizawa T, Suzuki H, Suzuki M, Takahashi M, Hibi T. Proton pump inhibitor-amoxicillin-clarithromycin versus proton pump inhibitor-amoxicillin-metronidazole as first-line *Helicobacter pylori* eradication therapy. J Clin Biochem Nutr. 2012;51:114–6.
2. Boyanova L, Mitov I. Geographic map and evolution of primary *Helicobacter pylori* resistance to antibacterial agents. Expert Rev Anti-Infect Ther. 2010;8:59–70.
3. Kim JM, Kim JS, Jung HC, Kim N, Kim YJ, Song IS. Distribution of antibiotic MICs for *Helicobacter pylori* strains over a 16-year period in patients from Seoul, South Korea. Antimicrob Agents Chemother. 2004;48:4843–7.

4. Lee JW, Kim N, Kim JM, Nam RH, Chang H, Kim JY, et al. Prevalence of primary and secondary antimicrobial resistance of *Helicobacter pylori* in Korea from 2003 through 2012. Helicobacter. 2013;18:206–14.
5. Kim JJ, Reddy R, Lee M, Kim JG, El-Zaatari FA, Osato MS, et al. Analysis of metronidazole, clarithromycin and tetracycline resistance of *Helicobacter pylori* isolates from Korea. J Antimicrob Chemother. 2001;47:459–61.
6. Duck WM, Sobel J, Pruckler JM, Song Q, Swerdlow D, Friedman C, et al. Antimicrobial resistance incidence and risk factors among *Helicobacter pylori*-infected persons, United States. Emerg Infect Dis. 2004;10:1088–94.
7. Hulten KG, Lamberth LB, Kalfus IN, Graham DY. National and regional US antibiotic resistance to *Helicobacter pylori*: lessons from a clinical trial. Gastroenterology. 2021;161:342–4.
8. Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, et al. *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut. 2013;62:34–42.
9. Megraud F, Bruyndonckx R, Coenen S, Wittkop L, Huang TD, Hoebeke M, et al. *Helicobacter pylori* resistance to antibiotics in Europe in 2018 and its relationship to antibiotic consumption in the community. Gut. 2021;70:1815–22.
10. Samra Z, Shmuely H, Niv Y, Dinari G, Passaro DJ, Geler A, et al. Resistance of *Helicobacter pylori* isolated in Israel to metronidazole, clarithromycin, tetracycline, amoxicillin and cefixime. J Antimicrob Chemother. 2002;49:1023–6.
11. Datta S, Chattopadhyay S, Patra R, De R, Ramamurthy T, Hembram J, et al. Most *Helicobacter pylori* strains of Kolkata in India are resistant to metronidazole but susceptible to other drugs commonly used for eradication and ulcer therapy. Aliment Pharmacol Ther. 2005;22:51–7.
12. Liu G, Xu X, He L, Ding Z, Gu Y, Zhang J, et al. Primary antibiotic resistance of *Helicobacter pylori* isolated from Beijing children. Helicobacter. 2011;16:356–62.
13. Kobayashi I, Murakami K, Kato M, Kato S, Azuma T, Takahashi S, et al. Changing antimicrobial susceptibility epidemiology of *Helicobacter pylori* strains in Japan between 2002 and 2005. J Clin Microbiol. 2007;45:4006–10.
14. Sukri A, Hanafiah A, Yusoff H, Nizam NAS, Nameyrra Z, Wong Z, et al. Multidrug-resistant *Helicobacter pylori* strains: a five-year surveillance study and its genome characteristics. Antibiotics (Basel). 2022;11:1391.
15. Lee JY, Kim N. Future trends of *Helicobacter pylori* eradication therapy in Korea. Korean J Gastroenterol. 2014;63:158–70.
16. Kim JY, Kim N, Kim SJ, Baik GH, Kim GH, Kim JM, et al. Regional difference of antibiotic resistance of *Helicobacter pylori* strains in Korea. Korean J Gastroenterol. 2011;57:221–9.
17. Lee JH, Ahn JY, Choi KD, Jung HY, Kim JM, Baik GH, et al. Nationwide antibiotic resistance mapping of *Helicobacter pylori* in Korea: a prospective multicenter study. Helicobacter. 2019;24:e12592.
18. Lee JY, Kim N, Kim MS, Choi YJ, Lee JW, Yoon H, et al. Factors affecting first-line triple therapy of *Helicobacter pylori* including CYP2C19 genotype and antibiotic resistance. Dig Dis Sci. 2014;59:1235–43.
19. Alarcón T, Domingo D, López-Brea M. Antibiotic resistance problems with *Helicobacter pylori*. Int J Antimicrob Agents. 1999;12:19–26.
20. Lerang F, Mourn B, Ragnhildstveit E, Sandvei PK, Tolås P, Whist JE, et al. Simplified 10-day bismuth triple therapy for cure of *Helicobacter pylori* infection: experience from clinical practice in a population with a high frequency of metronidazole resistance. Am J Gastroenterol. 1998;93:212–6.
21. Noach LA, Langenberg WL, Bertola MA, Dankert J, Tytgat GN. Impact of metronidazole resistance on the eradication of *Helicobacter pylori*. Scand J Infect Dis. 1994;26:321–7.
22. Logan RP, Gummett PA, Misiewicz JJ, Karim QN, Walker MM, Baron JH. One week eradication regimen for *Helicobacter pylori*. Lancet. 1991;338:1249–52.
23. Burette A, Glupczynski Y, De Prez C. Evaluation of various multi-drug eradication regimens for *Helicobacter pylori*. Eur J Gastroenterol Hepatol. 1992;4:817–23.
24. Rautelin H, Seppälä K, Renkonen OV, Vainio U, Kosunen TU. Role of metronidazole resistance in therapy of *Helicobacter pylori* infections. Antimicrob Agents Chemother. 1992;36:163–6.
25. Lerang F, Moum B, Haug JB, Tolås P, Breder O, Aubert E, et al. Highly effective twice-daily triple therapies for *Helicobacter pylori* infection and peptic ulcer disease: does in vitro metronidazole resistance have any clinical relevance? Am J Gastroenterol. 1997;92:248–53.
26. Thijs JC, Van Zwet AA, Thijs WJ, Van der Wouden EJ, Kooy A. One-week triple therapy with omeprazole, amoxycillin and tinidazole for *Helicobacter pylori* infection: the significance of imidazole resistance. Aliment Pharmacol Ther. 1997;11:305–9.
27. Harris AW, Pryce DI, Gabe SM, Karim QN, Walker MM, Langworthy H, et al. Lansoprazole, clarithromycin and metronidazole for seven days in *Helicobacter pylori* infection. Aliment Pharmacol Ther. 1996;10:1005–8.
28. Moayyedi P, Chalmers DM, Axon AT. Patient factors that predict failure of omeprazole, clarithromycin, and tinidazole to eradicate *Helicobacter pylori*. J Gastroenterol. 1997;32:24–7.
29. DeCross AJ, Marshall BJ, McCallum RW, Hoffman SR, Barrett LJ, Guerrant RL. Metronidazole susceptibility testing for *Helicobacter pylori*: comparison of disk, broth, and agar dilution methods and their clinical relevance. J Clin Microbiol. 1993;31:1971–4.
30. Van der Hulst RW, Van Der Ende A, Homan A, Roorda P, Dankert J, Tytgat GN. Influence of metroni-

dazole resistance on efficacy of quadruple therapy for *Helicobacter pylori* eradication. Gut. 1998;42:166–9.

31. Graham DY, Hoffman J, El-Zimaity HM, Graham DP, Osato M. Twice a day quadruple therapy (bismuth subsalicylate, tetracycline, metronidazole plus lansoprazole) for treatment of *Helicobacter pylori* infection. Aliment Pharmacol Ther. 1997;11:935–8.
32. Matsumoto S, Washizuka Y, Matsumoto Y, Tawara S, Ikeda F, Yokota Y, et al. Appearance of a metronidazole-resistant *Helicobacter pylori* strain in an infected-ICR-mouse model and difference in eradication of metronidazole-resistant and sensitive strains. Antimicrob Agents Chemother. 1997;41:2602–5.
33. Müller M, Lindmark DG. Uptake of metronidazole and its effect on viability in trichomonads and Entamoeba invadens under anaerobic and aerobic conditions. Antimicrob Agents Chemother. 1976;9:696–700.
34. Gatta L, Vakil N, Leandro G, Di Mario F, Vaira D. Sequential therapy or triple therapy for *Helicobacter pylori* infection: systematic review and meta-analysis of randomized controlled trials in adults and children. Am J Gastroenterol. 2009;104:3069–79.
35. Knight RC, Skolimowski IM, Edwards DI. The interaction of reduced metronidazole with DNA. Biochem Pharmacol. 1978;27:2089–93.
36. Jorgensen MA, Manos J, Mendz GL, Hazell SL. The mode of action of metronidazole in *Helicobacter pylori*: futile cycling or reduction? J Antimicrob Chemother. 1998;41:67–75.
37. Mann J. Medicinal compounds. In: Education in chemistry. Royal Society of Chemistry. 2008. http://www.rsc.org/Education/EiC/issues/2008May/MedicinalCompounds.asp. Accessed May 2008.
38. Solcà NM, Bernasconi MV, Piffaretti JC. Mechanism of metronidazole resistance in *Helicobacter pylori*: comparison of the *rdxA* gene sequences in 30 strains. Antimicrob Agents Chemother. 2000;44:2207–10.
39. Han F, Liu S, Ho B, Yan Z, Yan X. Alterations in *rdxA* and *frxA* genes and their upstream regions in metronidazole-resistant Helicobacter pylori isolates. Res Microbiol. 2007;158:38–44.
40. Zhang S, Wang X, Wise MJ, He Y, Chen H, Liu A, et al. Mutations of *Helicobacter pylori* RdxA are mainly related to the phylogenetic origin of the strain and not to metronidazole resistance. J Antimicrob Chemother. 2020;75:3152–5.
41. Lamichhane B, Wise MJ, Chua EG, Marshall BJ, Tay CY. A novel taxon selection method, aimed at minimizing recombination, clarifies the discovery of a new sub-population of *Helicobacter pylori* from Australia. Evol Appl. 2020;13:278–89.
42. Secka O, Berg DE, Antonio M, Corrah T, Tapgun M, Walton R, et al. Antimicrobial susceptibility and resistance patterns among *Helicobacter pylori* strains from The Gambia, West Africa. Antimicrob Agents Chemother. 2013;57:1231–7.
43. Jeong JY, Mukhopadhyay AK, Dailidiene D, Wang Y, Velapatino B, Gilman RH, et al. Sequential inactivation of *rdxA* (HP0954) and *frxA* (HP0642) nitroreductase genes causes moderate and high-level metronidazole resistance in *Helicobacter pylori*. J Bacteriol. 2000;182:5082–90.
44. Martinez-Júlvez M, Rojas AL, Olekhnovich I, Espinosa Angarica V, Hoffman PS, Sancho J. Structure of RdxA—an oxygen-insensitive nitroreductase essential for metronidazole activation in *Helicobacter pylori*. FEBS J. 2012;279:4306–17.
45. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The protein data bank. Nucleic Acids Res. 2000;28:235–42.
46. Berman H, Henrick K, Nakamura H. Announcing the worldwide protein data bank. Nat Struct Biol. 2003;10:980.
47. Chisholm SA, Owen RJ. Mutations in *Helicobacter pylori rdxA* gene sequences may not contribute to metronidazole resistance. J Antimicrob Chemother. 2003;51:995–9.
48. Kim SY, Joo YM, Lee HS, Chung IS, Yoo YJ, Merrell DS, et al. Genetic analysis of *Helicobacter pylori* clinical isolates suggests resistance to metronidazole can occur without the loss of functional *rdxA*. J Antibiot. 2009;62:43–50.
49. Tsugawa H, Suzuki H, Muraoka H, Ikeda F, Hirata K, Matsuzaki J, et al. Enhanced bacterial efflux system is the first step to the development of metronidazole resistance in *Helicobacter pylori*. Biochem Biophys Res Commun. 2011;404:656–60.
50. Lee SM, Kim N, Kwon YH, Nam RH, Kim JM, Park JY, et al. rdxA, frxA, and efflux pump in metronidazole-resistant *Helicobacter pylori*: their relation to clinical outcomes. J Gastroenterol Hepatol. 2018;33:681–8.
51. Chua EG, Debowski AW, Webberley KM, Peters F, Lamichhane B, Loke MF, et al. Analysis of core protein clusters identifies candidate variable sites conferring metronidazole resistance in *Helicobacter pylori*. Gastroenterol Rep (Oxf). 2019;7:42–9.

Part VII

Treatment

Synopsis of Antibiotic Treatment

43

David Y. Graham and Maria Pina Dore

Abstract

Helicobacter pylori (*H. pylori*) is a common infection responsible for considerable morbidity and mortality worldwide. It is now recommended that all *H. pylori* infections can be cured unless there are compelling reasons not to. Like other infectious diseases, the most reliable method to achieve high cure rates is to base therapy on the results of patient-specific susceptibility testing. Empiric therapy must be based on knowledge of the local susceptibility patterns tempered by data obtained from the patient concerning their antibiotic exposure. Data from clinical trials without susceptibility testing cannot be reliably used in other locations. Because of increasing resistance, triple therapies based on clarithromycin, metronidazole, or levofloxacin should only be used for susceptibility-based therapy. If there are no options other than to give empiric therapy, only those regimens proven to be effective locally should be used. Special efforts should be made to enhance patient adherence. In most regions, 14-day four-drug combinations are required for successful empiric therapy. Testing to confirm cure should be routinely done.

Keywords

Helicobacter pylori · Therapy
Susceptibility-based therapy · Patient compliance

43.1 Introduction

Although *Helicobacter pylori* (*H. pylori*) infections can result in peptic ulcer disease or gastric cancer (GC) and are one of the most common chronic bacterial infections of mankind, therapy remains a problem especially compared to other common infectious diseases. Treatment for most bacterial infections is based on knowledge of local antimicrobial susceptibility complemented by susceptibility testing of the organism infecting that particular patient. In contrast, the treatment for *H. pylori* has most often been chosen empirically without specific knowledge of the local susceptibility patterns or an attempt to obtain the resistance spectrum of the organism from the infected patient. Worldwide, the success rates of anti-*H. pylori* therapy are now generally poor and significantly inferior to what would be achieved by patient-specific susceptibility-based therapy [1, 2]. This is difficult to understand in that *H. pylori* can be cultured in vitro from biopsies and

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any hospital that has provisions for culture and susceptibility testing should be able to provide this service. Recently, next-generation sequencing has become available which allows susceptibility data to be obtained from fresh and formalin-fixed and paraffin-embedded gastric biopsies as well as from stools at least in the United States [3, 4].

After approximately 30 years of examining different therapies in thousands of patients, there is now sufficient data accumulated regarding the effectiveness of different regimens to allow rational susceptibility-based therapeutic decisions to be made and effectiveness to be estimated based on local susceptibility data [1, 5, 6].

43.2 Why Are *H. pylori* Infections So Difficult to Cure?

H. pylori occupy a number of different microenvironments within the stomach that vary from (a) residing within the highly acidic mucus on the gastric surface to (b) being attached to epithelial cells and forming a biofilm, or (c) residing within gastric epithelial cells. Each microenvironment offers different challenges for effective antimicrobial therapy. The intragastric milieu also experiences a wide range of changes in pH, volume, and composition throughout the day and with each meal and in relation to the gastric microbiota [7]. On average, most *H. pylori* appear to live in an environment where the average pH is approximately 3.5 [8–10]. At this pH, they do not replicate. Replication occurs between pH 6 and 7, such that many organisms are in environments where the bacteria are resistant to antimicrobials effective only during cell division such as amoxicillin [11]. In addition, the very large number of bacteria in the stomach essentially ensures that small populations of resistant strains will be present for those antibiotics in which the spontaneous rate of mutations in the genes responsible for resistance is relatively high [11–13].

Multidrug therapy is generally required to make the organism reach its different microenvironments and to prevent the development of resistance [12]. Because few antimicrobials are effective at low pH, successful therapy requires that gastric acid secretion be inhibited which also reduces dilution of the orally administered and topically active agents. Increasing the local pH to 6 encourages the organisms to divide and thus become susceptible to agents such as amoxicillin whose antimicrobial effect replicating organisms (e.g., the ability to build new cell walls).

43.3 Causes of Treatment Failure

The three most common reasons for treatment failure relate to problems with the organism (i.e., antimicrobial resistance), with the clinician (i.e., poor choice of drugs, doses, number of drug administrations, or treatment duration), or with the patient (i.e., failure to complete the regimen as prescribed due to side effects or failure to understand the importance of completing therapy).

43.4 Evidence-Based Therapy Is Susceptibility-Based Therapy

The frequent failure to link susceptibility data with the results of clinical trials resulted in the lack of data needed to understand the strengths and weaknesses of many treatment regimens [1]. As noted previously, many decades of trial-and-error and Hp-shmeta-analyses [14] were needed before sufficient susceptibility data had been acquired to allow clinicians to understand why therapies succeeded or failed and prospectively predict which regimen would most likely be effective for an individual patient, region, or society. A number of multidrug regimens are now available that will reliably cure 95% or more of compliant patients with susceptible infections [1] (Table 43.1).

The most commonly used anti-*H. pylori* antimicrobials worldwide are amoxicillin, clarithromycin, metronidazole (or tinidazole), tetracycline

Table 43.1 Recommended regimens for *Helicobacter pylori* eradication therapy

Treatment	Drugs, dosages, and duration
Empiric therapies	
Bismuth quadruple therapy b.i.d.	Bismuth subsalicylate or bismuth subcitrate 2 tablets q.i.d. and tetracycline hydrochloride (500 mg) b.i.d. plus metronidazole/tinidazole 500 mg, four times daily with meals and at bedtime and a PPI twice daily for 14 days
Bismuth quadruple therapy q.i.d.	Bismuth subsalicylate or bismuth subcitrate 2 tablets and tetracycline hydrochloride (500 mg) plus metronidazole/tinidazole (500 mg) four times daily with meals and at bedtime and a PPI twice daily for 14 days
Prepackaged bismuth quadruple therapy	PYLERA® and a PPI twice daily for 14 days
Rifabutin triple therapy	Rifabutin 150 mg twice daily, amoxicillin 1 g t.i.d. plus PPI (40 mg esomeprazole twice daily for 14 days)
Prepackaged rifabutin triple therapy	TALICIA® 4 capsules every 8 h for 14 days with food
Susceptibility-based therapies	
Triple therapy when *H. pylori* infection is proven to be susceptible to clarithromycin	Amoxicillin (1 g) and either clarithromycin (500 mg) or tinidazole (500 mg) or metronidazole (500 mg) plus a PPI all given twice daily for 14 days
Fluoroquinolone therapy when *H. pylori* is known to be susceptible to fluoroquinolones	Fluoroquinolone (e.g., levofloxacin 500 mg once daily), plus a PPI and amoxicillin 1 g twice daily for 14 days
Empiric salvage therapy	
Furazolidone quadruple therapy: with or without tetracycline	1. Bismuth subsalicylate or bismuth subcitrate 2 tablets and tetracycline hydrochloride (500 mg) both four times daily with meals and at bedtime plus furazolidone 100 mg t.i.d., with means and PPI twice daily for 14 days
	2. Bismuth subsalicylate or bismuth subcitrate 2 four times daily with meals and at bedtime plus furazolidone 100 mg and amoxicillin 1 g t.i.d., with meals plus a PPI twice daily for 14 days
Rifabutin triple therapy	Rifabutin 150 mg twice daily, amoxicillin 1 g t.i.d. plus PPI (40 mg esopeprazole twice daily for 14 days)
High-dose PPI-amoxicillin dual therapy	PPI (e.g., rabeprazole 40 mg, esomeprazole 40 mg) plus amoxicillin (500 mg or 750 mg) all four times daily at approximately 6 h intervals for 14 days
Obsolete therapies that should not be used because of antibiotic misuse	
Concomitant therapy	Clarithromycin (500 mg) and amoxicillin (1 g) **or** metronidazole (500 mg) plus PPI plus a PPI twice daily for 14 days
Sequential therapy	Amoxicillin (1 g) plus PPI standard dose twice daily for 5 days followed by clarithromycin (500 mg) and metronidazole or tinidazole (500 mg) plus PPI standard dose twice daily for additional 5 days
Hybrid therapy	Amoxicillin (1 g) plus PPI standard dose for twice daily 7 days followed by amoxicillin (500 mg), clarithromycin (500 mg), and metronidazole or tinidazole (500 mg) plus PPI standard dose twice daily for additional 7 days
Note about PPI dosing	In general, only second-generation PPIs should be used as they are more resistant to CYP2C19 metabolism. In western countries, we recommend 40 mg of esoprapzaole or rabeprazole b.i.d. in Asia 20 mg may be sufficient

PPI proton pump inhibitor

hydrochloride, one of the new-generation fluoroquinolones (e.g., levofloxacin), furazolidone, a bismuth salt, and rifabutin. These are generally prescribed in association with a gastric acid inhibitor, more commonly a proton pump inhibitor (PPI), or in alternative, a potassium-competitive acid blocker (P-CAB) such as vonoprazan that is still available only in some countries. Resistance does not develop to bismuth and rarely develops to amoxicillin, tetracycline, or furazolidone. Until recently, rifabutin was rarely used clinically except in the treatment of tuberculosis, and thus rates of resistance in the general population are low. In contrast, resistance to clarithromycin and fluoroquinolones has increased to where they should no longer be used as empiric therapy. Metronidazole resistance is high in most of the world except Japan and is unique in that the presence of resistance as identified by in vitro testing is not an "all or none" phenomenon and can be partially overcome by increasing the dose and duration of therapy [15, 16]. However, metronidazole remains effective when used as part of four-drug bismuth-containing therapy in which metronidazole resistance can often be completely overcome (see below) [16].

Failed therapy typically results in the development of resistance with clarithromycin, metronidazole, or fluoroquinolones. This most likely represents the selection of a preexisting minor population or resistant organism (heteroresistance). However, resistance to amoxicillin, tetracycline, or furazolidone occurs rarely following treatment failure, and these drugs can often be used again successfully.

43.5 General "Rules" for Choosing a Regimen

As with other infectious diseases, susceptibility-based therapy yields the best outcomes [1] and is the recommended approach. With essentially all current regimens, the cure rate is best when the drugs are administered for 14 days and the PPI is given as a double dose at least equivalent to 40 mg of omeprazole at least twice a day [5, 6, 17]. Overall, PPIs minimally affected by CYP2C19 metabolism are the best choice (e.g., esomeprazole or rabeprazole; we recommend 40 mg b.i.d.). Exceptions are discussed under each regimen. Single-drug and dual-drug therapies, with the exception of PPI plus amoxicillin dual therapy, have proven to provide overall poor cure rates in Western countries and are not recommended except where locally proven effective [18]. P-CABs such as vonoprazan have recently been introduced and are more effective than the average PPI; however, in most cases, they provide similar results to high-dose esomeprazole or rabeprazole (40 mg b.i.d., in Western countries, 20 mg in Asia). In the US/Europe, vonoprazan triple therapy and dual therapy (with amoxicillin) failed to achieve high cure rates. The reason for this unexpected result is as yet unclear. Nonetheless, it is probably best to refrain from their use until the regimens are optimized for use in the US/Europe.

43.6 *H. pylori* Therapies

43.6.1 Triple Therapies (Generally Only Used for Susceptibility-Based Therapy)

The basic regimen consisted of a PPI (e.g., at least 40 mg omeprazole or equivalent), amoxicillin (1 g), and a third drug (e.g., clarithromycin, metronidazole, or levofloxacin) twice a day for 14 days (Table 43.1). Shorter duration regimens provide lower cure rates with clarithromycin or metronidazole and typically fail to achieve even 90% cures with fluoroquinolones. For infections with strains susceptible to the antimicrobials chosen, one can anticipate that the regimen will reliably cure >90%, typically >95% of infections in patients adherent with the treatment [1]. In the last decade, clarithromycin triple therapy became obsolete because a high percentage of *H. pylori* strains had become resistant to clarithromycin. For example, in trials in which infected patients were treated with clarithromycin triple therapy, eradication rates were 90% and 22% for clarithromycin-sensitive strains and clarithromycin-resistant strains, respectively. Resistance to clarithromycin and fluoroquinolones cannot be overcome by increasing the dose or duration of therapy (i.e., it is all or none). Those patients with resistant infections effectively receive only two of the three drugs (e.g., clarithromycin is eliminated) and thus receive only a dual therapy with PPI plus amoxicillin. At the doses used in triple therapy, this dual therapy will cure less than 50% of patients. In Western countries, where rapid PPI metabolizers are frequent, cure rates generally fall to less than 20% (see Sect. 43.6.4). As noted previously, the cure rate with PPI, amoxicillin, and metronidazole triple therapy for 14 days with infections resistant to metronidazole can be as high as 75%, which is an unacceptable cure rate [15]. Triple therapies should be abandoned as empiric therapies. However, triple therapies can still remain excellent choices for use as susceptibility-based therapy. We suggest the substitution of amoxicillin with metronidazole only in penicillin-allergic individuals since metronidazole resistance is common and can reduce the efficacy of treatment.

43.6.2 Four-Drug Therapies

43.6.2.1 Bismuth Quadruple Therapy

The original four-drug therapy was the combination of a PPI, a bismuth, tetracycline HCl, and metronidazole (often called quadruple therapy or bismuth (subcitrate or subsalicylate) quadruple therapy) [19] (Table 43.1). Cure rates of >90%, often >95%, are expected when given for 14 days with good adherence. At least 50% of patients will experience side effects (e.g., nausea, abdominal pain, etc.), and poor adherence has often been the most important factor limiting its effectiveness [19]. As will be discussed below, the time invested in patient education, regarding side effects to expect, and the importance of completing therapy is time well spent. This regimen has proven to be highly effective despite metronidazole resistance, because resistance can often be overcome provided one uses full-dose metronidazole (e.g., 1500 or 1600 mg) and 14-day therapy [16, 19]. The regimen is complex requiring multiple pills to be given three or four times a day. Attempts to simplify this by packaging in blister packs and by combining several drugs into a single capsule have been made, but there are as yet no convincing data that this has translated into either improved compliance or outcome.

This regimen is often the first choice in patients unable to take penicillin or in areas where clarithromycin and metronidazole resistance precludes the use of empiric triple therapies. If the patient can take penicillin and metronidazole resistance is not present, there is no reason not to use a simpler and better-tolerated triple therapy. Bismuth quadruple therapy is also the first choice for retreatment (e.g., second-line therapy) following treatment failure with a regimen containing different antimicrobials.

Bismuth quadruple therapy is the least well-studied regimen in terms of comparing outcomes with susceptibility and compliance [19]. The simplified version (e.g., twice a day bismuth instead of four times a day for 10 days) may improve compliance and reduce side effects maintaining the same efficacy, at least in some specific regions [20]. Clearly, a well thought-out multi-region development program is needed to best understand and optimally utilize this regimen.

Alternate regimens are based on bismuth quadruple therapy, but replacing the metronidazole with furazolidone (100 mg t.i.d.) or tetracycline with amoxicillin (1 g t.i.d.) [21, 22].

43.6.2.2 Bismuth Furazolidone Quadruple Therapy

Although furazolidone is no longer widely available, it remains available in many regions where multidrug-resistant *H. pylori* are common such as in China and the Middle East. Furazolidone-tetracycline or furazolidone-amoxicillin quadruple therapies have proven to be highly effective despite the presence of *H. pylori* resistant to clarithromycin, metronidazole, and fluoroquinolones [21, 22]. The keys to successful therapy include

Table 43.2 Recommendations

1. Use susceptibility-based therapy whenever possible (based on culture or molecular methods)
2. Avoid clarithromycin, metronidazole, or fluoroquinolone-containing triple therapies unless the infection is proven to be susceptible to the antibiotics given
3. Therapy should be administered for 14 days
4. Patients must be educated about the importance of completing therapy and the nature of expected side effects
5. Confirmed cure should follow treatment, preferably with a locally validated noninvasive test such as a ^{13}C-urea breath test (at least 4 weeks after) or stool antigen test (at least 6 weeks after the end of therapy)

14-day therapy and full-dose tetracycline HCL and furazolidone (Table 43.2). Furazolidone (Furoxone) has proven effective as part of triple-drug therapies. Furazolidone is a synthetic nitrofuran with broad antibacterial activity based on interference with bacterial enzymes. It is a monoamine oxidase inhibitor (MAO inhibitor) and interacts with many drugs and foods (e.g., soy sauce, aged cheese, etc.) [23]. Side effects are common and patient instructions regarding food avoidances are needed [23]. Nonetheless, when given for 14 days, treatment failures are rarely making it the *piece de resistance* for therapy after multiple treatment failures in motivated patients.

43.6.3 Non-Bismuth Quadruple Therapies

This group consists of the combination of a PPI, amoxicillin, metronidazole/tinidazole, and clarithromycin given together or sequentially (concomitant therapy, sequential therapy, and hybrid sequential-concomitant therapy) [5, 6, 24, 25] (Table 43.1). The optimal duration is 14 days. None of these regimens are currently recommended as every patient receives at least one unneeded antibiotic and this has resulted in tens of thousands of kilograms of unnecessary antibiotics being administered/million therapies. The use of any of these four drugs clarithromycin and metronidazole-containing regimens is likely a major factor in the worldwide crisis of antibiotic misuse and increasing antimicrobial resistance.

43.6.3.1 Sequential Therapy (Generally Considered Obsolete Because of Antimicrobial Misuse)

Sequential therapy is now considered an obsolete regimen that was originally devised to overcome clarithromycin resistance [5, 6] by adding another drug (metronidazole). It consists of dual PPI-amoxicillin therapy followed by PPI-clarithromycin-metronidazole triple therapy. Originally, it was devised as a 5 + 5 or 10-day therapy and subsequently optimized as a 7 + 7 or 14-day therapy [26]. Its development is the poster child of how the trial-and-error methods failed as an effective means of developing a new therapeutic regimen [26]. Initially, sequential therapy appeared effective and that illusion was maintained by the primary emphasis focusing on showing it to be superior to triple therapy in regions where clarithromycin resistance had been proven to make triple therapy ineffective. Unfortunately, it was not recognized that the low prevalence of metronidazole resistance in the same regions hid the fact that the regimen was undermined by metronidazole resistance. The failure to routinely include susceptibility testing led to repetitive experiments in the same populations showing that indeed sequential therapy was superior to triple therapy (which was why it was originally developed). However, when used in Southern Italy or other regions, it often fails to provide acceptable cure rates [19]. Only then was it discovered that it was markedly less effective in the presence of metronidazole resistance. Moreover, randomized trials have also demonstrated lower eradication rates with sequential therapy as compared with clarithromycin triple therapy in Latin America and Asia. Although, according to the European Registry on *H. pylori* Management, sequential therapy is the most empirically prescribed first-line regimen in Italy, given its complexity and the lack of superiority to

14-day clarithromycin triple therapy in North America, sequential therapy has never been uniformly endorsed by expert Consensus as a first-line therapy.

43.6.3.2 Concomitant Therapy (Generally Considered Obsolete Because of Antimicrobial Misuse)

Concomitant therapy is the prime example of 4-drug clarithromycin and metronidazole containing regimens and should never be used. It is fundamentally identical to giving clarithromycin triple therapy and metronidazole triple therapy simultaneously. It is therefore effective in the presence of either clarithromycin or metronidazole resistance but becomes ineffective in the presence of dual clarithromycin-metronidazole resistance, which in most regions is rare. Treatment success is also duration-dependent and is optimal with 14-day therapy [5, 6, 24, 25]. Data from 19 randomized trials including 2070 European, Asian, and Latino American individuals showed that cure rates were significantly higher with concomitant quadruple therapy compared with clarithromycin triple therapy (90% and 78%, respectively). Concomitant therapy has previously been widely used as an empiric therapy; however, if susceptibility data showed the infection was sensitive, then a triple therapy would be more appropriate. One can estimate the prevalence of dual clarithromycin-metronidazole resistance by multiplying the known prevalence of each resistance rate. For example, in the Houston population, clarithromycin resistance and metronidazole resistance are approximately 15% and 25%, respectively, which preclude the use of either clarithromycin or metronidazole triple therapies. However, the prevalence of dual resistance is less than 5%, making empiric concomitant therapy an excellent empiric choice for treatment-naïve patients [1, 5, 6]. However, as noted above, this comes at a great cost measured in tons of unnecessary antibiotics being used, and is a major cause of antibiotic misuse.

43.6.3.3 Hybrid and Reverse Hybrid Therapy (Generally Considered Obsolete Because of Antimicrobial Misuse)

Hybrid therapy is a combination of sequential and concomitant therapy in which dual PPI-amoxicillin therapy is followed by all four drugs (PPI-amoxicillin-clarithromycin-metronidazole) as a 7 + 7 or 14-day therapy [27]. Reverse hybrid therapy reverses the order such that a 7-day concomitant regimen is followed by 7-day dual therapy. Both can be thought of as a 7-day concomitant therapy with either a dual-therapy lead-in or tail. This is another of the 4-drug clarithromycin metronidazole-containing regimens and although the amount of unneeded antibiotic is less than that with concomitant therapy, it should never be used as its success is based on antibiotic misuse.

43.6.4 PPI-Amoxicillin High-Dose Dual Therapy

As noted above, if the intragastric pH can be maintained reliably at 6 or greater, it is theoretically possible to overcome dormancy, encouraging *H. pylori* to replicate making them highly susceptible to amoxicillin. The pharmacokinetics of amoxicillin suggest that if given every 6 h (a maximum of 8 h is allowed at night), amoxicillin will be present whenever the organism becomes susceptible, making it possible to achieve >90% cure rates with 14-day dual therapy [28]. Dual therapy was of great interest in the early to mid-1990s but eventually failed in that reliable cures above 80% could not be achieved. The importance of pH control has become increasingly well-documented [28]. The effectiveness of this approach has been repeatedly demonstrated in Asia where a high proportion of the population consists of slow or moderate PPI metabolizers (e.g., related to CYP2C19 polymorphisms), plus a high prevalence of corpus gastritis, both of which promote high pH with PPI therapy [29, 30]. The use of PPIs that are not highly metabolized by the CYP2C19 pathway (e.g., rabeprazole or esomeprazole) or by a new selective potassium-competitive acid blocker allows to achieve this high pH consistently. Higher doses of more rapidly metabolized PPIs are needed than when using slowly metabolized PPIs. In this regimen, amoxicillin is given at 500 or 750 mg every 6 h. However, this has not yet proven to be a reliable strategy in the West where rapid and ultrarapid PPI metabolizers are common. The duration of 14 days has proven to be the best. It is also recommended that acid food and drinks (e.g., soft drinks) be prohibited during treatment [31]. If reliable pH control can be ensured with one of the new selective P-CABs, it may be possible to reduce the frequency of administration of amoxicillin to twice or three times a day. For example, in studies conducted in the United States and Korea, eradication rates in treatment-naive patients were 72% and 79%, respectively. However, in a randomized trial conducted in China including naive patients assigned to high-dose dual therapy or bismuth quadruple therapy, there was no significant difference in eradication rates between the two groups. Results of clinical trials in Japan have shown that either vonoprazan or esomeprazole plus amoxicillin dual therapy achieves high and equivalent cure rates and is non-inferior to vonoprazan triple therapy in the same population. In other countries, effectiveness has varied despite increasing the amoxicillin dosage and the duration of therapy. We recommend the therapy only in regions where it has been proven to be reliably highly effective. Generally, efforts to optimize dual therapy are still lacking.

However with high-dose, dual therapy remains the concern of *Clostridium difficile* infection, especially in the elderly, the majority of *H. pylori*-infected individuals in high-resources countries.

43.7 Patient Education to Enhance Adherence

Noncompliance with recommended drug therapy has long been a recognized issue impeding successful treatment since the time of Hippocrates. It has been suggested that the term “compliance,” which implies obedience, be replaced with “adherence”. According to the WHO, adherence can be defined as the degree to which the person’s behavior corresponds with the agreed recommendations from a health care provider [32]. Adherence reflects the active participation of both the doctor and patient in their commitment to the treatment chosen. Adherence is an especially important problem with long-term therapies for chronic conditions such as treatment of HIV infections or tuberculosis. Most *H. pylori* eradication therapies are short (i.e., 2 weeks), but are frequently associated with minor but noticeable side effects. Poor adherence is one of the major causes of low eradication rates and thus an important problem. The considerable literature regarding adherence focuses on features such as the doctor-patient relationship (i.e., trust), the importance of education about the goals and benefits of therapy, and the adverse effects of treatment failure (e.g., risk of GC, recurrence of peptic ulcer, etc.) [32–35]. Factors that have proven important in predicting reduced adherence include the complexity of the regimen (e.g., the number of medications, dosing intervals, duration of therapy, relation with meals, etc.), the expected side effects (e.g., bad taste in the mouth with clarithromycin), and the importance of tailoring the education about the regimens to the age, sex, ethnicity, educational level, cultural, and socioeconomic background of the individual patient [32–35].

Based on the successful program used by de Boer when working with bismuth quadruple therapy [36], we recommend establishing a trust-based doctor-patient relationship including:

(a) Taking a detailed medical history and adequate time for follow-up visits
(b) Explaining in simplistic terms the effects of the infection on the stomach, the potential outcomes of the infection, and how cure of the infection results in healing of the damage, prevention of ulcers and ulcer recurrences, reducing or eliminating the risk of GC and, eventually, of anemia, thrombocytopenia, and vitamin B12 deficiency

(c) Providing a description of the complexities of the regimen chosen, the necessity for adherence to the treatment schedule, and completing the regimen, including a commitment to try
(d) Providing a clear description of the medications and plan for dosing provided in writing and, if possible, provide appropriate containers (pill boxes or blister packs when possible) arranged according to the dosing plans in relation to meals and bedtime
(e) Providing a description of the adverse effects, such as feeling unwell (nausea, headaches, taste disturbances, loose stools, etc.), which is an expected consequence of treatment

Adherence should be monitored by recalls and by counting the remaining pills, which are asked to be returned by the patient at the end of the treatment.

It has not been clearly defined what rate of treatment completion defines "sufficient" compliance, and it most likely varies according to the type of regimen chosen as well as the individual and/or population rate of antibiotic resistance. In a previous study, once the cutoff for completion of the prescribed bismuth subsalicylate, metronidazole, and tetracycline triple therapy was set at 60%, 96% of those having taken their medications at above this rate yielded successful *H. pylori* eradication vs. only 69% of the rest [37]. Lee and colleagues [38] performed a randomized controlled trial using this cutoff to evaluate an enhanced compliance program in which 125 patients received 2 weeks of bismuth metronidazole tetracycline triple therapy. The program included pharmacist counseling and written information on the infection, the effect of eradication, follow-up phone calls, and supplied medication calendars and pill boxes and their instructions for use. Those enrolled in the adherence program had a slight improvement in adherence defined as completing more than 60% of their treatment compared to controls (e.g., 95% vs. 89%). However, raising the cutoff point to 90% resulted in a statistically significant difference in eradication rates (i.e., 89% vs. 67%; $p < 0.01$). Importantly, 20% refused to participate.

Another randomized trial included 76 patients receiving non-bismuth-based (lansoprazole, amoxicillin, and clarithromycin) triple therapies, half of whom received counseling, written educational leaflets, compliance charts, and follow-ups [35, 38, 39]. Adherence to treatment occurred in 92.1% of those receiving training vs. 23.7% in controls when the cutoff point was set at 100%. *H. pylori* eradication rates were 94.7% vs. 73.7%, respectively, favoring training where 100% of those with successful eradication had taken more than 65% of their medications. Pharmacoeconomic analysis deemed the intervention cost-effective. Collectively, patient counseling and follow-up represent time well-spent in relation to treatment success. Despite this, a recent study reported patient experiences related to *H. pylori* management were predominantly negative [40].

43.8 Recommendations

The most reliable method and highest cure rates are obtained by basing therapy on the results of patient-specific susceptibility testing (Table 43.2). All empiric therapy must be based on knowledge of the local susceptibility patterns tempered by data obtained from the patient concerning their antibiotic exposure. Clinical trials without provisions for susceptibility testing or without stopping rules to limit exposure to poor regimens should not be done. Without susceptibility testing, the trial fails to provide useful information except for the population treated in the trial, and without stopping rules, the risk exposure would be excessive. If there are no options other than to give empiric therapy, only those regimens proven to be effective locally should be used.

References

1. Graham DY. *Helicobacter pylori* update: gastric cancer, reliable therapy, and possible benefits. Gastroenterology. 2015;148:719–31.e3.
2. Graham DY, Fischbach L. *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. Gut. 2010;59:1143–53.

3. Dore MP, Graham DY. Modern approach to the diagnosis of *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2022;55 Suppl 1:S14–21.
4. Graham DY, Moss SF. Antimicrobial susceptibility testing for *Helicobacter pylori* is now widely available: when, how, why. Am J Gastroenterol. 2022;117:524–8.
5. Graham DY, Lee YC, Wu MS. Rational *Helicobacter pylori* therapy: evidence-based medicine rather than medicine-based evidence. Clin Gastroenterol Hepatol. 2014;12:177–86, e3; Discussion e12–3.
6. Wu JY, Liou JM, Graham DY. Evidence-based recommendations for successful *Helicobacter pylori* treatment. Expert Rev Gastroenterol Hepatol. 2014;8:21–8.
7. Eun CS, Kim BK, Han DS, Kim SY, Kim KM, Choi BY, et al. Differences in gastric mucosal microbiota profiling in patients with chronic gastritis, intestinal metaplasia, and gastric cancer using pyrosequencing methods. Helicobacter. 2014;19:407–16.
8. Marcus EA, Inatomi N, Nagami GT, Sachs G, Scott DR. The effects of varying acidity on *Helicobacter pylori* growth and the bactericidal efficacy of ampicillin. Aliment Pharmacol Ther. 2012;36:972–9.
9. Sachs G, Weeks DL, Wen Y, Marcus EA, Scott DR, Melchers K. Acid acclimation by *Helicobacter pylori*. Physiology. 2005;20:429–38.
10. Scott DR, Marcus EA, Wen Y, Oh J, Sachs G. Gene expression in vivo shows that *Helicobacter pylori* colonizes an acidic niche on the gastric surface. Proc Natl Acad Sci U S A. 2007;104:7235–40.
11. Graham DY, Shiotani A. New concepts of resistance in the treatment of *Helicobacter pylori* infections. Nat Clin Pract Gastroenterol Hepatol. 2008;5:321–31.
12. Graham DY. Antibiotic resistance in *Helicobacter pylori*: implications for therapy. Gastroenterology. 1998;115:1272–7.
13. Laxminarayan R, Bhutta Z, Duse A, Jenkins P, O'Brien T, Okeke IN, et al. Drug resistance. In: Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB, et al., editors. Disease control priorities in developing countries. 2nd ed. Washington, DC: World Bank; 2006. p. 1031–52.
14. Graham DY, Dore MP. *Helicobacter pylori* therapy: a paradigm shift. Expert Rev Anti-Infect Ther. 2016;14:577–85.
15. Bardhan K, Bayerdörffer E, Veldhuyzen van Zanten SJ, Lind T, Mégraud F, Delchier JC, et al. The HOMER study: the effect of increasing the dose of metronidazole when given with omeprazole and amoxicillin to cure *Helicobacter pylori* infection. Helicobacter. 2000;5:196–201.
16. Fischbach L, Evans EL. Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for *Helicobacter pylori*. Aliment Pharmacol Ther. 2007;26:343–57.
17. Rimbara E, Fischbach LA, Graham DY. Optimal therapy for *Helicobacter pylori* infections. Nat Rev Gastroenterol Hepatol. 2011;8:79–88.
18. Graham DY, Liou JM. Primer for development of guidelines for *Helicobacter pylori* therapy using antimicrobial stewardship. Clin Gastroenterol Hepatol. 2022;20:973–83.e1.
19. Graham DY, Lee SY. How to effectively use bismuth quadruple therapy: the good, the bad, and the ugly. Gastroenterol Clin N Am. 2015;44:537–63.
20. Dore MP, Farina V, Cuccu M, Mameli L, Massarelli G, Graham DY. Twice-a-day bismuth-containing quadruple therapy for *Helicobacter pylori* eradication: a randomized trial of 10 and 14 days. Helicobacter. 2011;16:295–300.
21. Lu H, Zhang W, Graham DY. Bismuth-containing quadruple therapy for *Helicobacter pylori*: lessons from China. Eur J Gastroenterol Hepatol. 2013;25:1134–40.
22. Liang X, Xu X, Zheng Q, Zhang W, Sun Q, Liu W, et al. Efficacy of bismuth-containing quadruple therapies for clarithromycin-, metronidazole-, and fluoroquinolone-resistant *Helicobacter pylori* infections in a prospective study. Clin Gastroenterol Hepatol. 2013;11:802–7.e1.
23. Graham DY, Lu H. Furazolidone in *Helicobacter pylori* therapy: misunderstood and often unfairly maligned drug told in a story of French bread. Saudi J Gastroenterol. 2012;18:1–2.
24. Essa AS, Kramer JR, Graham DY, Treiber G. Meta-analysis: four-drug, three-antibiotic, non-bismuth-containing "concomitant therapy" versus triple therapy for *Helicobacter pylori* eradication. Helicobacter. 2009;14:109–18.
25. Gisbert JP, Calvet X. Review article: non-bismuth quadruple (concomitant) therapy for eradication of *Helicobacter pylori*. Aliment Pharmacol Ther. 2011;34:604–17.
26. Liou JM, Chen CC, Chen MJ, Chen CC, Chang CY, Fang YJ, et al. Sequential versus triple therapy for the first-line treatment of *Helicobacter pylori*: a multicentre, open-label, randomised trial. Lancet. 2013;381:205–13.
27. Hsu PI, Wu DC, Wu JY, Graham DY. Modified sequential *Helicobacter pylori* therapy: proton pump inhibitor and amoxicillin for 14 days with clarithromycin and metronidazole added as a quadruple (hybrid) therapy for the final 7 days. Helicobacter. 2011;16:139–45.
28. Furuta T, Graham DY. Pharmacologic aspects of eradication therapy for *Helicobacter pylori* infection. Gastroenterol Clin N Am. 2010;39:465–80.
29. Labenz J, Stolte M, Blum AL, Jorias I, Leverkus F, Sollböhmer M, et al. Intragastric acidity as a predictor of the success of *Helicobacter pylori* eradication: a study in peptic ulcer patients with omeprazole and amoxicillin. Gut. 1995;37:39–43.
30. Labenz J, Leverkus F, Börsch G. Omeprazole plus amoxicillin for cure of *Helicobacter pylori* infection. Factors influencing the treatment success. Scand J Gastroenterol. 1994;29:1070–5.
31. Yang JC, Lin CJ, Wang HL, Chen JD, Kao JY, Shun CT, et al. High-dose dual therapy is superior to stan-

dard first-line or rescue therapy for *Helicobacter pylori* infection. Clin Gastroenterol Hepatol. 2015;13:895–905.e5.

32. World Health Organization. Adherence to long-term therapies: evidence for action. Geneva: World Health Organization; 2003.
33. Osterberg L, Blaschke T. Adherence to medication. N Engl J Med. 2005;353:487–97.
34. Eraker SA, Kirscht JP, Becker MH. Understanding and improving patient compliance. Ann Intern Med. 1984;100:258–68.
35. O'Connor JP, Taneike I, O'Morain C. Improving compliance with *Helicobacter pylori* eradication therapy: when and how? Ther Adv Gastroenterol. 2009;2:273–9.
36. de Boer WA. How to achieve a near 100% cure rate for *H. pylori* infection in peptic ulcer patients. A personal viewpoint. J Clin Gastroenterol. 1996;22:313–6.
37. Graham DY, Lew GM, Malaty HM, Evans DG, Evans DJ Jr, Klein PD, et al. Factors influencing the eradication of *Helicobacter pylori* with triple therapy. Gastroenterology. 1992;102:493–6.
38. Lee M, Kemp JA, Canning A, Egan C, Tataronis G, Farraye FA. A randomized controlled trial of an enhanced patient compliance program for *Helicobacter pylori* therapy. Arch Intern Med. 1999;159:2312–6.
39. Al-Eidan FA, McElnay JC, Scott MG, McConnell JB. Management of *Helicobacter pylori* eradication—the influence of structured counselling and follow-up. Br J Clin Pharmacol. 2002;53:163–71.
40. Shah SC, Bonnet K, Schulte R, Peek RM Jr, Schlundt D, Roumie CL. *Helicobacter pylori* management is associated with predominantly negative patient experiences: results from a focused qualitative analysis. Dig Dis Sci. 2022;67:4387–94.

44 Triple Therapy

Ju Yup Lee

Abstract

Triple therapy has long been used as the first-line therapy for *Helicobacter pylori* (*H. pylori*) infection, but its efficacy has been continuously decreasing over the past 10 years. The main cause of decrease in the *H. pylori* eradication rate of conventional triple therapy is increasing resistance of *H. pylori* to clarithromycin. The *H. pylori*-associated factors affecting the eradication rate of conventional triple therapy are antibiotic resistance, colonization density of *H. pylori* in the gastric mucosal membrane, and virulence factors of *cagA* and *vacA*. Host factors are medication adherence, excess gastric acid secretion, cytochrome P450 gene polymorphism (CYP2C19), treatment duration, drug side effects, underlying gastroduodenal disease, gastritis pattern, smoking, diabetes, obesity, etc. Due to decrease in the *H. pylori* eradication rate of the triple therapy, the current standard first-line treatment for *H. pylori* infection, it has become necessary to develop a new first-line treatment capable of increasing the *H. pylori* eradication rate.

Keywords

Triple therapy · Clarithromycin · Amoxicillin Resistance

44.1 Introduction

Triple therapy has been considered worldwide the standard first-line treatment for *Helicobacter pylori* (*H. pylori*) infection. After the first presentation of the proton pump inhibitor (PPI)-based triple therapy using the combination of two antibiotics (amoxicillin and clarithromycin) in 1997 by the European Helicobacter Study Group in Maastricht as an *H. pylori* eradication therapy [1], it has been used worldwide, including the USA and Europe, as the first-line *H. pylori* eradication therapy. In South Korea as well, triple therapy has been recommended as the first-line treatment for *H. pylori* eradication therapy as the 1998 official recommendation [2, 3]. Also, the latest revised version (2013) of the guidelines specifies that in the current situation in which no therapy better than the conventional triple therapy has yet been developed, the conventional triple therapy is recommended as the standard first-line treatment [4]. However, the recent trend of decrease in the eradication rate with the standard triple therapy due to increasing antibiotic resistance necessitates urgent countermeasures [5]. This work is intended to investigate the eradication rate change patterns of conventional triple therapy, reasons for declining treatment success, and factors influencing the eradication rate of triple therapy.

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44.2 Current Status of the Eradication Rates of Standard Triple Therapy

The eradication rates of triple therapy in its earlier years were recognized to be high, ranging between 80 and 90%. However, with increasing antibiotic resistance, its eradication rate has been continuously decreasing. According to the previous reports, including meta-analyses, only ~18% exceed the eradication rate of 85% on an intention-to-treat (ITT) analysis, with ~60% falling short of 80% [6]. As such, in Western Europe, standard triple therapy is by now considered "legacy therapy" [6]. In fact, European guidelines recommend it as the first-line treatment only when the prevalence of clarithromycin resistance is under 20% [7].

The situation is not different in South Korea. A study reviewing 19 Korean domestic papers between 2005 and 2013 reported that the eradication rate of standard triple therapy was 90% or higher in 1997–1998, but kept decreasing afterward, reaching the level of under 80% in 2009 and 75.8% in 2012 [8]. In contrast, other studies reported that no great differences were observed in the eradication rate of standard triple therapy for the past 5–11 years [9–11]. Such discrepancies are considered to be attributable to the regional differences in antibiotic resistance or different definition criteria for eradication [9–14]. Taking all these regional differences into consideration, a meta-analysis was performed with a total of 104 studies (38 randomized studies and 66 observational studies) published in South Korea between 1998 and 2013, including abstracts, on standard triple therapy in first-line treatment covering 42,124 patients. The eradication rate on an ITT analysis was 74.6% (95% confidence interval [CI], 72.1–77.2%) and on a per-protocol (PP) analysis 82.0% (95% CI, 80.8–83.2%), with both analyses showing a significantly decreasing trend over 16 years from 1998 to 2013 (Fig. 44.1). Additionally, the eradication rates of 7- and 14-day triple regimens were 81.1 and 85.3% on a PP analysis, respectively [12].

In recent evidence-based guideline, selected randomized controlled trials (RCTs) conducted in Korea which have used standard triple therapy since 2007 and 26 studies were included in meta-analysis [15]. Overall, pooled eradication rates of standard triple therapy derived from these studies were 71.6% (95% CI, 69.9–73.3%) in ITT analysis and 79.6% (95% CI, 76.6–82.2%) in PP analysis (Fig. 44.2). Studies were divided into 2007–2011 and 2012–2016. Pooled eradication rates of 2007–2011 and 2012–2016 were 72.3% (95% CI, 71.2–74.4%) and 70.3% (95% CI, 68.4–72.1%) in ITT analysis, respectively. The pooled eradication rate of standard triple therapy in 2012–2016 was on the decline compared with 2007–2011 [15].

From these results, it may be concluded that despite regional differences, the overall trend clearly demonstrates that the efficacy of standard triple therapy has been decreasing in South Korea, which suggests the necessity for establishing a new efficient standard treatment.

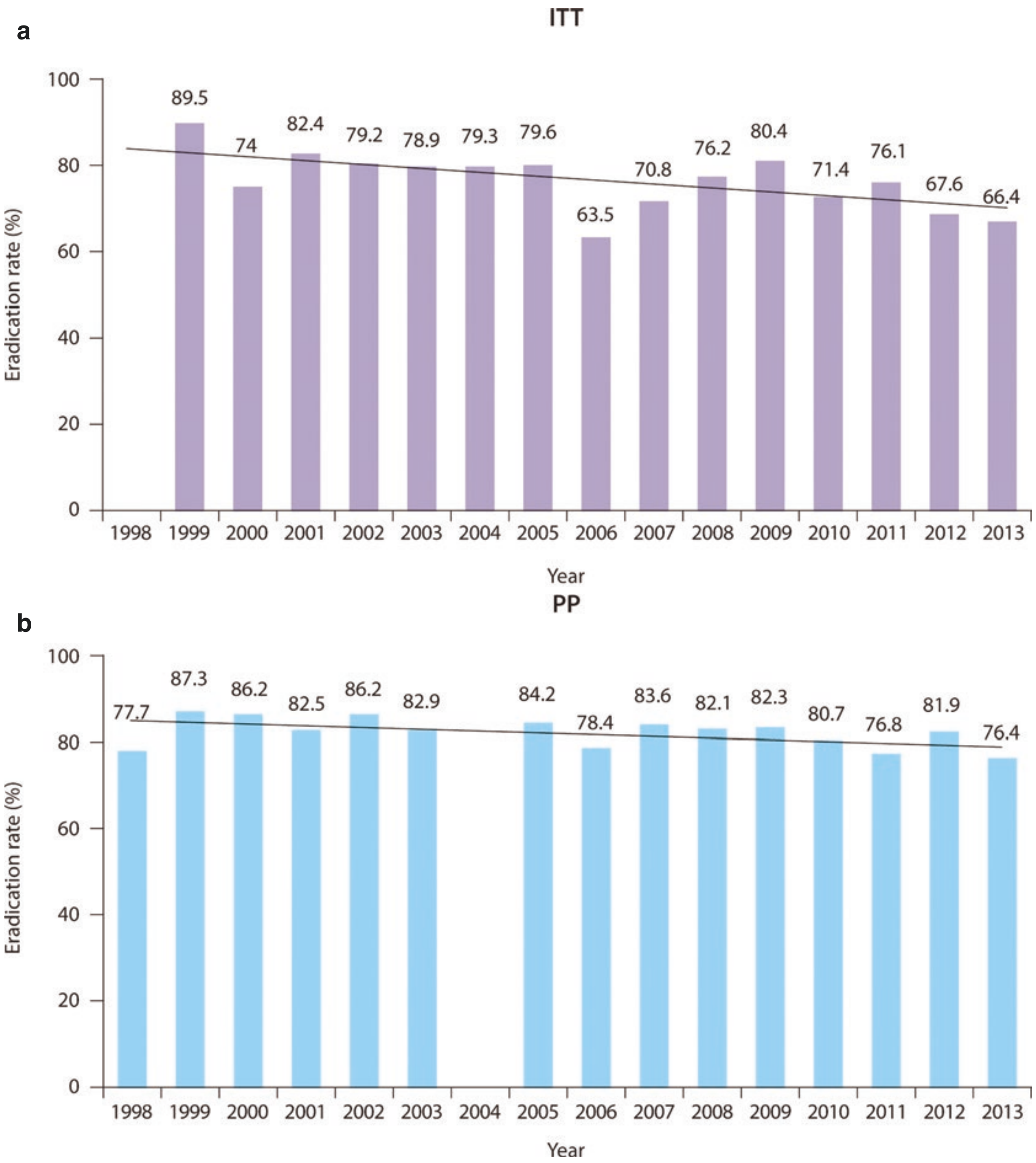
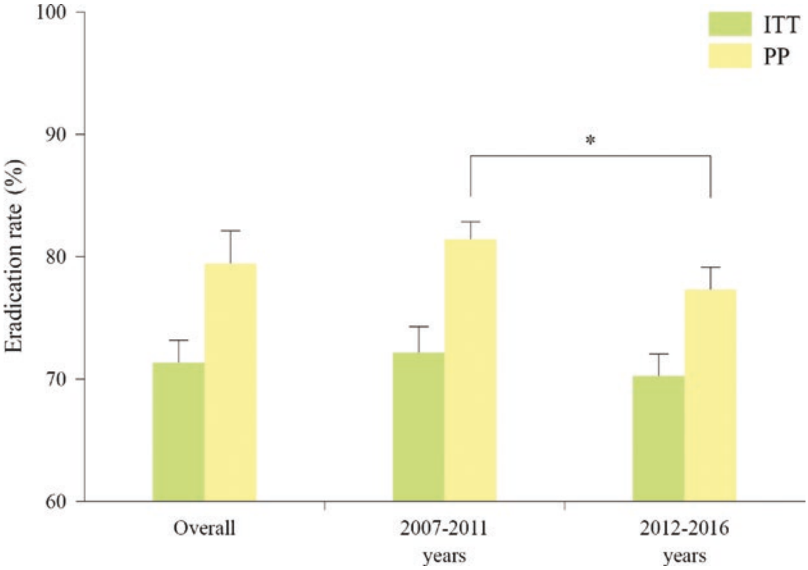


Fig. 44.1 Latest 16-year trends of eradication rate of standard triple therapy as reported in a meta-analysis in South Korea. On both ITT analysis (**a**) and PP analysis (**b**), significant decreases in eradication rate are observed ($p < 0.001$ for ITT, $p = 0.003$ for PP). *ITT* intention to treat, *PP* per protocol. (Modified from Gong et al. [12])

Fig. 44.2 Time trends of pooled *Helicobacter pylori* eradication rates of standard triple therapy from randomized controlled trials performed in Korea (by years). *ITT* intention-to-treat, *PP* per protocol. *$p < 0.01$. (Adapted from Jung et al. [15])



44.3 Factors Influencing the Eradication Rate of Standard Triple Therapy

With the eradication rate of standard triple therapy decreasing, many research efforts have been put to identify the reasons for this phenomenon. Factors that may affect eradication rate can be largely categorized into *H. pylori* factors and host factors. Table 44.1 lists the factors influencing eradication rate identified so far [16]. Of them, the factor reported to be the main cause of eradication failure is antibiotic resistance, clarithromycin resistance in particular [17–21].

44.3.1 *H. pylori* Factors

44.3.1.1 Antibiotic Resistance

For the past two decades, clarithromycin has been used to treat bacterial infections affecting the respiratory system, and the decrease in eradication rate due to increasing prevalence of clarithromycin resistance of *H. pylori* has been reported globally. As such, clarithromycin resistance is the main cause of eradication failure [17–21].

Table 44.1 Factors influencing the *H. pylori* eradication therapy

Host factors	*H. pylori* factors
Medication adherence	Antibiotic resistance
Excess gastric acid secretion	Density of *H. pylori* in the stomach
Cytochrome P450 gene polymorphism	*cagA* status
Treatment duration	*vacA* alleles status
Drug side effects	*dupA* status
Underlying gastroduodenal disease (PUD vs. NUD)	Bacterial coccoid forms
Gastritis pattern (antral vs. pangastritis)	
Smoking	
Diabetics	
Obesity	

H. pylori Helicobacter pylori, *PUD* peptic ulcer disease, *NUD* non-ulcer dyspepsia
Adapted from Zullo et al. [16]

Despite regional differences, many regions in the USA, Europe, and advanced countries in Asia exhibit high clarithromycin resistance of 20% or higher. In particular, central, western, and southern European countries exhibit clarithromycin resistance rates exceeding 20% nationwide [22]. A multicenter study in Japan also reported the clarithromycin resistance rate in 2002 to be 18.9%, which rapidly increased to 27.2% in 2006

[23]. The European average amoxicillin resistance rate is reported to be lower than 2% [24–26], while those in Africa, Asia, and Latin America are reported to be ranging between 6 and 59% [27, 28]. The *H. pylori* resistance rates toward primary antibiotics since 2000 were over 20% for clarithromycin, over 40% for metronidazole, and over 10% for quinolone in South Korea [29]. Looking more closely at the clarithromycin resistance rates in South Korea, the strains isolated in Hanyang University Hospital and Seoul National University Hospital in 1987 and 1994 were as low as 0% and 2.8%, respectively, which rapidly increased to 13.8% in 2003 [30]. The resistance rates of strains isolated in Seoul National University Bundang Hospital, which was opened in 2003, rapidly increased from 22.9% (2003–2005) to 25.5% (2006–2008) and 37.0% (2009–2012) [31]. As presented above, the antibiotic resistance of *H. pylori* strains, especially clarithromycin-resistant strains, is continuously increasing, acting as the major cause of failure of *H. pylori* eradication with standard triple therapy.

44.3.1.2 Other *H. pylori* Factors

High colonization density of *H. pylori* in the gastric mucosal membrane is associated with decrease in eradication rate [32–34], and coccoid form *H. pylori* can also decrease eradication rate due to its low antimicrobial susceptibility [35, 36]. Eradication rate is also affected by the *H. pylori* virulence factor *cagA*. A meta-analysis estimated that the eradication rate in the *cagA*-negative *H. pylori* is lower than that in the *cagA*-positive *H. pylori* by 11% (95% CI, 3–19%; $p = 0.011$) [37]. Moreover, *vacA* s1m1 allele is known to have higher antimicrobial susceptibility than *vacA* s2m2 allele [38]. Shiota et al. [39] reported that another virulence factor called duodenal ulcer promoting gene (*dupA*) is an independent risk factor for eradication failure.

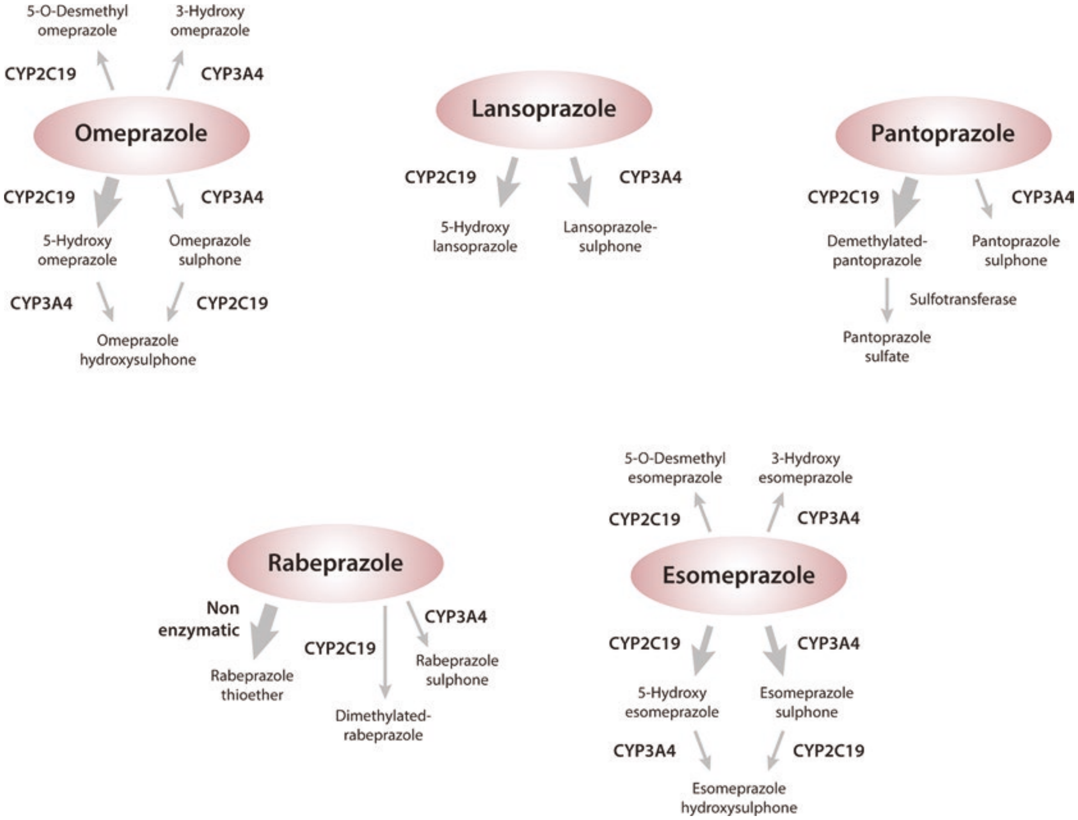
44.3.2 Host Factors

44.3.2.1 Medication Adherence

A low medication adherence of the patient is one of the important factors for eradication failure because the maximum efficacy of antibiotics relies on the administration of adequate dose for an optimal period of time [40]. Since triple therapy involves the concurrent medication of three different drugs, medication adherence rate tends to fall due to more frequent occurrence of side effects. Graham et al. [6] reported that side effects compel about 12% of patients to stop medication [6]. Therefore, physicians should provide patients with detailed information about side effects that may occur during medication and recommend the continuation of medication in case of mild side effects. Meta-analyses estimated the patient compliance rate of standard triple therapy at 94–96.8%, which is very high [41, 42]. If patient compliance rate is lower than 80%, eradication is very likely to fail, and the prevalence of antibiotic resistance is also likely to increase accordingly [43].

44.3.2.2 Excess Gastric Acid Secretion

Gastric acidity regulation is an important factor for *H. pylori* eradication because insufficient inhibitory regulation of gastric acid destabilizes antibiotics such as clarithromycin or amoxicillin, thereby decreasing their eradication rates. An adequate inhibition of gastric acid being an important factor for eradication success, CYP2C19 genotype can be a determinant factor for the success in eradication of the PPI-based standard triple therapy. PPI is known to be usually metabolized by CYP2C19 via the hepatic cytochrome p450 system [44] (Fig. 44.3). CYP2C19 is classified into extensive metabolizer (EM), intermediate metabolizer (IM), and poor metabolizer (PM) depending on gene polymorphism. PMs are known to have about 20-fold bioavailability compared to EMs owing to extremely slow PPI metabolism [45]. Asians and Caucasians have remarkably different CYP2C19 genotypes, and PM accounts for only 3–4% among Caucasians and African Americans, 14% among Koreans and Chinese, and 22.3% among Japanese [45, 46]. According to a meta-analysis of three previous studies on CYP2C19 genotypes, omeprazole was influenced by CYP2C19 genotypes, but rabeprazole was not [47–49]. In a more recent meta-analysis of randomized clinical studies, PMs showed higher eradication rates than


5-O-Desmethyl omeprazole
3-Hydroxy omeprazole
CYP2C19
CYP3A4
Omeprazole
CYP2C19
CYP3A4
5-Hydroxy omeprazole
Omeprazole sulphone
CYP3A4
CYP2C19
Omeprazole hydroxysulphone
Lansoprazole
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5-Hydroxy lansoprazole
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Demethylated-pantoprazole
Pantoprazole sulphone
Sulfotransferase
Pantoprazole sulfate
Rabeprazole
Non enzymatic
CYP2C19
CYP3A4
Rabeprazole thioether
Rabeprazole sulphone
Dimethylated-rabeprazole
5-O-Desmethyl esomeprazole
3-Hydroxy esomeprazole
CYP2C19
CYP3A4
Esomeprazole
CYP2C19
CYP3A4
5-Hydroxy esomeprazole
Esomeprazole sulphone
CYP3A4
CYP2C19
Esomeprazole hydroxysulphone

Fig. 44.3 Metabolic pathway related to cytochrome P450 isoenzyme (CYP2C19 and CYP3A4) according to the types of proton pump inhibitor (PPI). (Adapted from Sugimoto and Furuta [44])

EMs, and while eradication rate was influenced by the CYP2C19 genotype under omeprazole and lansoprazole medication, it was not the case under rabeprazole and esomeprazole medication [50]. While the eradication rate of triple therapy using lansoprazole and rabeprazole was not influenced by CYP2C19 genotype in a South Korean study [51], other studies reported that the eradication rate was significantly higher in PMs compared to EMs in triple therapy including pantoprazole and esomeprazole [14, 52]. However, it is not easy to examine CYP2C19 genotype in clinical settings. Therefore, regulating the type and dose of PPI was presented as a more practical approach in the second Asia-Pacific Consensus Guideline [53].

Potassium-competitive acid blockers (P-CABs) inhibit gastric acid secretion via selective and reversible inhibition of H^+/K^+-ATPase. Since P-CABs compete with K^+, their activity is dose-dependent. Moreover, the onset of P-CAB activity is faster than that of PPI activity; moreover, acid suppression by P-CABs is of longer duration, making the latter group of drugs more potent therapies for *H. pylori* eradication; furthermore, because P-CABs are acid-stable and less impaired by the CYP2C19 system than PPIs [54].

Recently, Zhang et al. [55] conducted meta-analysis based on RCTs for the efficacy and safety of P-CABs and PPIs for *H. pylori* eradication and 7 studies and 1168 patients were included. The pooled eradication rate determined by ITT analysis was 90.2% for P-CABs -based and 75.5% for PPI-based triple therapy (pooled RR 1.17; 95% CI, 1.08–1.28; $p < 0.001$). The PP analysis also demonstrated significant superiority of P-CABs (pooled eradication rate 92.4% vs. 77.8%; pooled RR 1.14; 95% CI, 1.03–1.26; $p < 0.01$). However, most included studies were conducted in Japan and the authors concluded that the efficacy of P-CAB-based triple therapy is superior to that of PPI-based triple therapy as a first-line approach to *H. pylori* eradication, particularly in Japanese patients.

In 2022, Choi et al. [56] report the efficacy of tegoprazan as a part of first-line triple therapy for *H. pylori* eradication. A randomized, double-blind, controlled, multicenter study was performed to evaluate whether a 50 mg tegoprazan-based triple therapy was non-inferior to a 30 mg lansoprazole-based triple therapy. A total of 350 patients were randomly allocated and the *H. pylori* eradication rates in the tegoprazan and lansoprazole groups were 62.86% and 60.57% in an ITT analysis and 69.33% and 67.33% in a PP analysis, respectively (non-inferiority test, $p = 0.0090$ and $p = 0.0127$). Although the results showed that tegoprazan was non-inferior to lansoprazole, the overall eradication rate was less than 70% in both groups and tegoprazan seems not to overcome the clarithromycin resistance of *H. pylori*. Further Korean studies are needed to clarify whether tegoprazan-based therapy is more effective than PPI-based therapy for *H. pylori* eradication.

44.3.2.3 Treatment Duration

In order to counteract the declining eradication rate, extension of eradication treatment duration to a 14-day regimen was considered; Calvet et al. [57] performed a meta-analysis and reported that duration of extension increased the eradication rates. The increase in eradication rate through extended eradication treatment in 10- and 14-day regimens was 4% and 6%, respectively, according to reports in countries including the USA. According to some other reports, however, extending the duration of eradication treatment yielded unfavorable results in terms of cost-effectiveness, and due to the increase in side effects through the prolonged medication and subsequent decrease in medication adherence, no statistically significant difference in eradication rate was yielded by the extension [58, 59]. Looking at the eradication rates according to the treatment duration in South Korea, a prospective multicenter study conducted in 2007 yielded the finding that the duration extension from 7 to 14 days did not result in significant increase in eradication rate [60], whereas a meta-analysis performed in 2008 estimated the increase in eradication rate at about 10% as the treatment duration was prolonged from 7 to 14 days [61].

A recent evidence-based Korean guideline analyzed the eradication rate of 7-day, 10-day, and 14-day standard triple therapy. The pooled eradication rate of 7-day standard triple therapy was 70.0% (95% CI, 68.5–71.4%) and that of 10-day therapy was 73.7% (95% CI, 69.8–77.2%) in ITT analysis. The pooled eradication rate (ITT) of 14-day therapy was 78.1% (95% CI, 75.2–80.7%) which was significantly higher than those of 7-day and 10-day therapy ($p < 0.01$ for both duration). Eradication rates between the 7-day and 10-day therapy were not significantly different. Based on the above analysis and the available evidences, a 14-day therapy is recommended when considering standard triple therapy as a first-line treatment without clarithromycin resistance test [13].

However, negative views prevail as to the effect of extended triple therapy in overcoming clarithromycin resistance. Clarithromycin eradicates *H. pylori* by binding to ribosome, and resistance impedes the binding to ribosome. Consequently, the question may be raised whether this deficit can be improved by increasing the administration dose and duration [8, 62].

44.3.2.4 Drug Side Effects

Zullo et al. [41] performed a meta-analysis and estimated the incidence of side effects of standard triple therapy at 9.8% and the rate of discontinuation of medication due to severe side effects at 0.007%. Tong et al. investigated seven studies in a meta-analysis and reported the incidence of side effects of standard triple therapy to be approximately 8.7% (6.0–17.1%) [63]. Side effects of standard triple therapy are bloating, heartburn, loss of appetite, altered taste (bitter taste in the mouth), nausea and vomiting, abdominal pain, headache, indigestion, diarrhea, constipation, itching, etc. The most recent meta-analysis performed in South Korea reviewed 41 studies (24 randomized clinical studies and 17 observational studies) covering 8018 patients and estimated the incidence of drug side effects at 24.0%, with the most common side effect being altered taste, followed by soft stool or diarrhea, abdominal discomfort, and nausea. The cases of discontinuing eradication treatment due to severe side effects accounted for 1.81%, demonstrating that most patients endure well side effects [12].

44.3.2.5 Underlying Gastroduodenal Disease

According to some reports, patients with non-ulcer (functional) dyspepsia exhibit lower eradication rates than patients with peptic ulcer disease [64]. This difference is ascribable to different *H. pylori* strains involved. Dyspeptic patients are easily infected by strains with low virulence (*cagA*-negative; *vacA* s2 or m2 genotype) and low proliferation rate. Such strains are known to have low antimicrobial susceptibility. Patients with non-ulcer dyspepsia are also reported to exhibit higher clarithromycin resistance rates than patients with peptic ulcer disease, but there is no consensus about this point [22]. There is also a report that patients with non-ulcer dyspepsia exhibit higher incidence of *H. pylori* reinfection compared to peptic ulcer patients, and this has yet to be clarified in further research [65].

44.3.2.6 Gastritis Patterns

Pattern of *H. pylori*-associated gastritis is also one of the factors affecting eradication rate. It was reported that pangastritis was associated with lower eradication rate compared to antral gastritis, which did not change when the standard triple therapy was extended to 14 days [66, 67].

44.3.2.7 Smoking

Many studies pointed out that smoking is associated with *H. pylori* eradication failure. A meta-analysis reviewing 22 clinical studies covering 5538 patients [68] reported that compared to nonsmokers, smokers showed higher potential for treatment failure in 7-day standard triple therapy, estimating the difference at 8.4% [68]. Smoking reduces blood flow in the stomach and mucus secretion, increases gastric acid secretion, and changes the specific metabolic activity of cytochrome P450 isoenzyme involved in PPI metabolism, and all these physiological changes are considered to contribute to increasing the eradication failure rate [68]. However, smoking-related decrease in eradication rate appeared under clarithromycin-amoxicillin medication, but not under clarithromycin-tinidazole medication [69]. Additionally, no noticeable smoking-related decrease in eradication rate was observed in 14-day triple therapy or sequential therapy [70].

44.3.2.8 Other Factors

Some studies reported that chronic diseases affected treatment outcomes, such as low eradication rate in diabetic patients [71], high clarithromycin resistance in patients with chronic renal diseases [72], and high eradication rate in patients with chronic liver diseases [73], but the effects of such chronic diseases on eradication rate are not consistent in these studies and still lack evidence.

Body mass index (BMI) can also affect eradication rate, and a report on obesity (BMI >25) was associated with decreased eradication rate with 7-day standard triple therapy [74].

Several studies reported that social drinking is associated with eradication success. Drinking alcohol of moderate alcohol content (5–40%) decreases gastric acid secretion and has antimicrobial effect on *H. pylori* and strengthens the prostaglandin-mediated mucosal defense, thus contributing to *H. pylori* eradication [75–78]. In most studies, however, alcohol intake did not influence eradication rate [79].

The association of *H. pylori* eradication rate with age is different from study to study. Some studies reported that aged patients showed higher eradication rate, ascribing it to the increase in intestinal metaplasia and gastric mucosal atrophy with increasing age [80–83]. Specifically, intestinal metaplasia creates an adverse environment for *H. pylori*, leading to decreased colonization density, which is more favorable for eradication, and gastric mucosal atrophy decreases gastric acid secretion, thus promoting the antimicrobial activity of the drug that increases in high pH. In contrast, a study on patients with functional dyspepsia or peptic ulcer disease who underwent standard triple therapy reported that the older patent group (≥45 years) showed significantly lower *H. pylori* eradication rate [21]. The mechanism of decreasing eradication rate in elderly patients has not yet been known, but there is a report that eradication rate is lowered in case of many comorbidities [84].

44.4 Conclusions

Standard triple therapy has long been the first-line treatment for *H. pylori* infection, but its eradication rate has been continuously decreasing for the past 15 years. The major cause of eradication rate is increase in clarithromycin resistance. There is a need for establishing a new first-line treatment capable of increasing eradication rate. Extending the treatment period to 14 days should be considered, and follow-up studies of P-CAB in *H. pylori* eradication treatment should be expected.

References

1. Malfertheiner P, Megraud F, O'Morain C, Bell D, Bianchi Porro G, Deltenre M, et al. Current European concepts in the management of *Helicobacter pylori* infection – the Maastricht consensus report. The European *Helicobacter pylori* study group (EHPSG). Eur J Gastroenterol Hepatol. 1997;9:1–2.
2. Korean H. pylori Study Group. Diagnosis and treatment of *Helicobacter pylori* infection in Korea. Korean J Gastroenterol. 1998;32:275–89.
3. Kim N, Kim JJ, Choe YH, Kim HS, Kim JI, Chung IS. Diagnosis and treatment guidelines for *Helicobacter pylori* infection in Korea. Korean J Gastroenterol. 2009;54:269–78.
4. Kim SG, Jung HK, Lee HL, Jang JY, Lee H, Kim CG, et al. Guidelines for the diagnosis and treatment of *Helicobacter pylori* infection in Korea, 2013 revised edition. J Gastroenterol Hepatol. 2014;29:1371–86.
5. Lee JY, Kim N. Future trends of *Helicobacter pylori* eradication therapy in Korea. Korean J Gastroenterol. 2014;63:158–70.
6. Graham DY, Fischbach L. *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. Gut. 2010;59:1143–53.
7. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of *Helicobacter pylori* infection – the Maastricht IV/Florence consensus report. Gut. 2012;61:646–64.
8. Heo J, Jeon SW. Changes in the eradication rate of conventional triple therapy for *Helicobacter pylori* infection in Korea. Korean J Gastroenterol. 2014;63:141–5.
9. Jung YS, Lee SH, Park CS, Oh MJ, Kim KO, Jang BI, et al. Trends in the eradication rates of *Helicobacter pylori* infection in Daegu and Gyeongsangbuk-do, Korea: multicenter study over 13 years. Korean J Gastroenterol. 2014;63:82–9.
10. Yoon KH, Park SW, Lee SW, Kim BJ, Kim JG. Clarithromycin-based standard triple therapy can still be effective for *Helicobacter pylori* eradication in some parts of the Korea. J Korean Med Sci. 2014;29:1240–6.
11. Yoon JH, Baik GH, Sohn KM, Kim DY, Kim YS, Suk KT, et al. Trends in the eradication rates of *Helicobacter pylori* infection for eleven years. World J Gastroenterol. 2012;18:6628–34.
12. Gong EJ, Yun SC, Jung HY, Lim H, Choi KS, Ahn JY, et al. Meta-analysis of first-line triple therapy for *Helicobacter pylori* eradication in Korea: is it time to change? J Korean Med Sci. 2014;29:704–13.
13. Kim JY, Kim NY, Kim SJ, Baik GH, Kim GH, Kim JM, et al. Regional difference of antibiotic resistance of *Helicobacter pylori* strains in Korea. Korean J Gastroenterol. 2011;57:221–9.
14. Lee JY, Kim N, Kim MS, Choi YJ, Lee JW, Yoon H, et al. Factors affecting first-line triple therapy of *Helicobacter pylori* including CYP2C19 genotype and antibiotic resistance. Dig Dis Sci. 2014;59:1235–43.
15. Jung HK, Kang SJ, Lee YC, Yang HJ, Park SY, Shin CM, et al. Evidence-based guidelines for the treatment of *Helicobacter pylori* infection in Korea 2020. Gut Liver. 2021;15:168–95.
16. Zullo A, De Francesco V, Hassan C. Predicting *Helicobacter pylori* eradication: how to teach an old dog new tricks! J Clin Gastroenterol. 2012;46:259–61.
17. Nishizawa T, Suzuki H, Suzuki M, Takahashi M, Hibi T. Proton pump inhibitor-amoxicillin-clarithromycin versus proton pump inhibitor-amoxicillin-metronidazole as first-line *Helicobacter pylori* eradication therapy. J Clin Biochem Nutr. 2012;51:114–6.
18. Oh HS, Lee DH, Seo JY, Cho YR, Kim N, Jeoung SH, et al. Ten-day sequential therapy is more effective than proton pump inhibitor-based therapy in Korea: a prospective, randomized study. J Gastroenterol Hepatol. 2012;27:504–9.
19. Basu PP, Rayapudi K, Pacana T, Shah NJ, Krishnaswamy N, Flynn M. A randomized study comparing levofloxacin, omeprazole, nitazoxanide, and doxycycline versus triple therapy for the eradication of *Helicobacter pylori*. Am J Gastroenterol. 2011;106:1970–5.
20. Bigard MA, Delchier JC, Riachi G, Thibault P, Barthelemy P. One-week triple therapy using omeprazole, amoxycillin and clarithromycin for the

eradication of *Helicobacter pylori* in patients with non-ulcer dyspepsia: influence of dosage of omeprazole and clarithromycin. Aliment Pharmacol Ther. 1998;12:383–8.

21. Perri F, Villani MR, Festa V, Quitadamo M, Andriulli A. Predictors of failure of *Helicobacter pylori* eradication with the standard 'Maastricht triple therapy'. Aliment Pharmacol Ther. 2001;15:1023–9.
22. De Francesco V, Giorgio F, Hassan C, Manes G, Vannella L, Panella C, et al. Worldwide *H. pylori* antibiotic resistance: a systematic review. J Gastrointestin Liver Dis. 2010;19:409–14.
23. Kobayashi I, Murakami K, Kato M, Kato S, Azuma T, Takahashi S, et al. Changing antimicrobial susceptibility epidemiology of *Helicobacter pylori* strains in Japan between 2002 and 2005. J Clin Microbiol. 2007;45:4006–10.
24. Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, et al. *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut. 2013;62:34–42.
25. Toracchio S, Marzio L. Primary and secondary antibiotic resistance of *Helicobacter pylori* strains isolated in central Italy during the years 1998–2002. Dig Liver Dis. 2003;35:541–5.
26. Boyanova L. Prevalence of multidrug-resistant *Helicobacter pylori* in Bulgaria. J Med Microbiol. 2009;58:930–5.
27. Asrat D, Kassa E, Mengistu Y, Nilsson I, Wadstrom T. Antimicrobial susceptibility pattern of *Helicobacter pylori* strains isolated from adult dyspeptic patients in Tikur Anbassa University Hospital, Addis Ababa, Ethiopia. Ethiop Med J. 2004;42:79–85.
28. Nahar S, Mukhopadhyay AK, Khan R, Ahmad MM, Datta S, Chattopadhyay S, et al. Antimicrobial susceptibility of *Helicobacter pylori* strains isolated in Bangladesh. J Clin Microbiol. 2004;42:4856–8.
29. Boyanova L, Mitov I. Geographic map and evolution of primary *Helicobacter pylori* resistance to antibacterial agents. Expert Rev Anti-Infect Ther. 2010;8:59–70.
30. Kim JM, Kim JS, Jung HC, Kim N, Kim YJ, Song IS. Distribution of antibiotic MICs for *Helicobacter pylori* strains over a 16-year period in patients from Seoul, South Korea. Antimicrob Agents Chemother. 2004;48:4843–7.
31. Lee JW, Kim N, Kim JM, Nam RH, Chang H, Kim JY, et al. Prevalence of primary and secondary antimicrobial resistance of *pylori* in Korea from 2003 through 2012. Helicobacter. 2013;18:206–14.
32. Perri F, Clemente R, Festa V, Quitadamo M, Conoscitore P, Niro G, et al. Relationship between the results of pre-treatment urea breath test and efficacy of eradication of *Helicobacter pylori* infection. Ital J Gastroenterol Hepatol. 1998;30:146–50.
33. Lai YC, Yang JC, Huang SH. Pre-treatment urea breath test results predict the efficacy of *Helicobacter pylori* eradication therapy in patients with active duodenal ulcers. World J Gastroenterol. 2004;10:991–4.
34. Molnar B, Szoke D, Ruzsovics A, Tulassay Z. Significantly elevated *Helicobacter pylori* density and different genotype distribution in erosions as compared with normal gastric biopsy specimen detected by quantitative real-time PCR. Eur J Gastroenterol Hepatol. 2008;20:305–13.
35. Aggarwal N, Snyder P, Owens SR. Unusual *Helicobacter pylori* in gastric resection specimens: an old friend with a new look. Int J Surg Pathol. 2011;19:297–302.
36. Andersen LP, Rasmussen L. *Helicobacter pylori*-coccoid forms and biofilm formation. FEMS Immunol Med Microbiol. 2009;56:112–5.
37. Suzuki T, Matsuo K, Sawaki A, Ito H, Hirose K, Wakai K, et al. Systematic review and meta-analysis: importance of CagA status for successful eradication of *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2006;24:273–80.
38. van Doorn LJ, Schneeberger PM, Nouhan N, Plaisier AP, Quint WG, de Boer WA. Importance of *Helicobacter pylori* cagA and vacA status for the efficacy of antibiotic treatment. Gut. 2000;46:321–6.
39. Shiota S, Nguyen LT, Murakami K, Kuroda A, Mizukami K, Okimoto T, et al. Association of *Helicobacter pylori* dupA with the failure of primary eradication. J Clin Gastroenterol. 2012;46:297–301.
40. De Francesco V, Ierardi E, Hassan C, Zullo A. *Helicobacter pylori* therapy: present and future. World J Gastrointest Pharmacol Ther. 2012;3:68–73.
41. Zullo A, De Francesco V, Hassan C, Morini S, Vaira D. The sequential therapy regimen for *Helicobacter pylori* eradication: a pooled-data analysis. Gut. 2007;56:1353–7.
42. Jafri NS, Hornung CA, Howden CW. Meta-analysis: sequential therapy appears superior to standard therapy for *Helicobacter pylori* infection in patients naive to treatment. Ann Intern Med. 2008;148:923–31.
43. Vakil N, Vaira D. Treatment for *H. pylori* infection: new challenges with antimicrobial resistance. J Clin Gastroenterol. 2013;47:383–8.
44. Sugimoto M, Furuta T. Efficacy of esomeprazole in treating acid-related diseases in Japanese populations. Clin Exp Gastroenterol. 2012;5:49–59.
45. Dickson EJ, Stuart RC. Genetics of response to proton pump inhibitor therapy: clinical implications. Am J Pharmacogenomics. 2003;3:303–15.
46. Chong E, Ensom MH. Pharmacogenetics of the proton pump inhibitors: a systematic review. Pharmacotherapy. 2003;23:460–71.
47. Padol S, Yuan Y, Thabane M, Padol IT, Hunt RH. The effect of CYP2C19 polymorphisms on *H. pylori* eradication rate in dual and triple first-line PPI therapies: a meta-analysis. Am J Gastroenterol. 2006;101:1467–75.
48. McNicholl AG, Linares PM, Nyssen OP, Calvet X, Gisbert JP. Meta-analysis: esomeprazole or rabeprazole vs. first-generation pump inhibitors in the treatment of *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2012;36:414–25.

49. Zhao F, Wang J, Yang Y, Wang X, Shi R, Xu Z, et al. Effect of CYP2C19 genetic polymorphisms on the efficacy of proton pump inhibitor-based triple therapy for *Helicobacter pylori* eradication: a meta-analysis. Helicobacter. 2008;13:532–41.
50. Tang HL, Li Y, Hu YF, Xie HG, Zhai SD. Effects of CYP2C19 loss-of-function variants on the eradication of *H. pylori* infection in patients treated with proton pump inhibitor-based triple therapy regimens: a meta-analysis of randomized clinical trials. PLoS One. 2013;8:e62162.
51. Lee JH, Jung HY, Choi KD, Song HJ, Lee GH, Kim JH. The influence of CYP2C19 polymorphism on eradication of *Helicobacter pylori*: a prospective randomized study of lansoprazole and rabeprazole. Gut Liver. 2010;4:201–6.
52. Kang JM, Kim N, Lee DH, Park YS, Kim JS, Chang IJ, et al. Effect of the CYP2C19 polymorphism on the eradication rate of *Helicobacter pylori* infection by 7-day triple therapy with regular proton pump inhibitor dosage. J Gastroenterol Hepatol. 2008;23:1287–91.
53. Fock KM, Katelaris P, Sugano K, Ang TL, Hunt R, Talley NJ, et al. Second Asia-Pacific consensus guidelines for *Helicobacter pylori* infection. J Gastroenterol Hepatol. 2009;24:1587–600.
54. Park JY, Kim JG. New *Helicobacter pylori* eradication therapies. Korean J Gastroenterol. 2018;72:237–44.
55. Zhang M, Pang M, Zhang M. Efficacy and safety of potassium-competitive acid blockers versus proton pump inhibitors as *Helicobacter pylori* eradication therapy: a meta-analysis of randomized clinical trials. Clinics (Sao Paulo). 2022;77:100058.
56. Choi YJ, Lee YC, Kim JM, Kim JI, Moon JS, Lim YJ, et al. Triple therapy-based on tegoprazan, a new potassium-competitive acid blocker, for first-line treatment of *Helicobacter pylori* infection: a randomized, double-blind, phase III, clinical trial. Gut Liver. 2022;16:535–46.
57. Calvet X, Garcia N, Lopez T, Gisbert JP, Gene E, Roque M. A meta-analysis of short versus long therapy with a proton pump inhibitor, clarithromycin and either metronidazole or amoxycillin for treating *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2000;14:603–9.
58. Fuccio L, Minardi ME, Zagari RM, Grilli D, Magrini N, Bazzoli F. Meta-analysis: duration of first-line proton-pump inhibitor based triple therapy for *Helicobacter pylori* eradication. Ann Intern Med. 2007;147:553–62.
59. Kang JH, Lim YJ. Trend for treatment of *Helicobacter pylori* in overseas country. Korean J Helicobacter Up Gastrointest Res. 2012;12:224–31.
60. Kim BG, Lee DH, Ye BD, Lee KH, Kim BW, Kim SG, et al. Comparison of 7-day and 14-day proton pump inhibitor-containing triple therapy for *Helicobacter pylori* eradication: neither treatment duration provides acceptable eradication rate in Korea. Helicobacter. 2007;12:31–5.
61. Kang BK, Kim BW. *Helicobacter pylori* virulence factors and host factors. Korean J Helicobacter Up Gastrointest Res. 2011;11:7–12.
62. Heo J, Jeon SW. Optimal treatment strategy for *Helicobacter pylori*: era of antibiotic resistance. World J Gastroenterol. 2014;20:5654–9.
63. Tong JL, Ran ZH, Shen J, Xiao SD. Sequential therapy vs. standard triple therapies for *Helicobacter pylori* infection: a meta-analysis. J Clin Pharm Ther. 2009;34:41–53.
64. Gisbert JP, Marcos S, Gisbert JL, Pajares JM. *Helicobacter pylori* eradication therapy is more effective in peptic ulcer than in non-ulcer dyspepsia. Eur J Gastroenterol Hepatol. 2001;13:1303–7.
65. Zullo A, Rinaldi V, Hassan C, Taggi F, Giustini M, Winn S, et al. Clinical and histologic predictors of *Helicobacter pylori* infection recurrence. J Clin Gastroenterol. 2000;31:38–41.
66. Zullo A, Severi C, Vannella L, Hassan C, Sbrozzi-Vanni A, Annibale B. Role of gastritis pattern on *Helicobacter pylori* eradication. Intern Emerg Med. 2012;7:517–22.
67. Severi C, Abdullahi M, Tari R, Vannella L, Marcheggiano A, Capoccia D, et al. High efficacy of bismuth subcitrate for *Helicobacter pylori* eradication in pangastritis. Dig Liver Dis. 2009;41:555–8.
68. Suzuki T, Matsuo K, Ito H, Sawaki A, Hirose K, Wakai K, et al. Smoking increases the treatment failure for *Helicobacter pylori* eradication. Am J Med. 2006;119:217–24.
69. Namiot DB, Leszczynska K, Namiot Z, Kurylonek AJ, Kemona A. Smoking and drinking habits are important predictors of *Helicobacter pylori* eradication. Adv Med Sci. 2008;53:310–5.
70. De Francesco V, Zullo A, Margiotta M, Marangi S, Burattini O, Berloco P, et al. Sequential treatment for *Helicobacter pylori* does not share the risk factors of triple therapy failure. Aliment Pharmacol Ther. 2004;19:407–14.
71. Ojetti V, Pellicano R, Fagoonee S, Migneco A, Berrutti M, Gasbarrini A. *Helicobacter pylori* infection and diabetes. Minerva Med. 2010;101:115–9.
72. Aydemir S, Boyacioglu S, Gur G, Demirbilek M, Can FK, Korkmaz M, et al. *Helicobacter pylori* infection in hemodialysis patients: susceptibility to amoxicillin and clarithromycin. World J Gastroenterol. 2005;11:842–5.
73. Jung SW, Lee SW, Hyun JJ, Kim DI, Koo JS, Yim HJ, et al. Efficacy of *Helicobacter pylori* eradication therapy in chronic liver disease. Dig Liver Dis. 2009;41:134–40.
74. Abdullahi M, Annibale B, Capoccia D, Tari R, Lahner E, Osborn J, et al. The eradication of *Helicobacter pylori* is affected by body mass index (BMI). Obes Surg. 2008;18:1450–4.
75. Brenner H, Rothenbacher D, Bode G, Adler G. Relation of smoking and alcohol and coffee consumption to active *Helicobacter pylori* infection: cross sectional study. BMJ. 1997;315:1489–92.

76. Weisse ME, Eberly B, Person DA. Wine as a digestive aid: comparative antimicrobial effects of bismuth salicylate and red and white wine. BMJ. 1995;311:1657–60.
77. Marimon JM, Bujanda L, Gutierrez-Stampa MA, Cosme A, Arenas JI. In vitro bactericidal effect of wine against *Helicobacter pylori*. Am J Gastroenterol. 1998;93:1392.
78. Kuepper-Nybelen J, Thefeld W, Rothenbacher D, Brenner H. Patterns of alcohol consumption and *Helicobacter pylori* infection: results of a population-based study from Germany among 6545 adults. Aliment Pharmacol Ther. 2005;21:57–64.
79. Nam TM, Lee DH, Kang KP, Lee JH, Chung JI, Choi HC, et al. Clinical factors that potentially affect the treatment outcome of *Helicobacter pylori* eradication therapy with using a standard triple regimen in peptic ulcer patients. Korean J Gastrointest Endosc. 2008;36:200–5.
80. Treiber G, Ammon S, Klotz U. Age-dependent eradication of *Helicobacter pylori* with dual therapy. Aliment Pharmacol Ther. 1997;11:711–8.
81. Broutet N, Tchamgoue S, Pereira E, Lamouliatte H, Salamon R, Megraud F. Risk factors for failure of *Helicobacter pylori* therapy – results of an individual data analysis of 2751 patients. Aliment Pharmacol Ther. 2003;17:99–109.
82. Cutler AF, Schubert TT. Patient factors affecting *Helicobacter pylori* eradication with triple therapy. Am J Gastroenterol. 1993;88:505–9.
83. Labenz J, Leverkus F, Borsch G. Omeprazole plus amoxicillin for cure of *Helicobacter pylori* infection. Factors influencing the treatment success. Scand J Gastroenterol. 1994;29:1070–5.
84. Byun YH, Jo YJ, Kim SC, Lee JS, Shin WY, Park YS, et al. Clinical factors that predicts successful eradication of *Helicobacter pylori*. Korean J Gastroenterol. 2006;48:172–9.

Quadruple Therapy

45

Ju Yup Lee

Abstract

Bismuth quadruple therapy (BQT) has been used as the second-line rescue therapy in cases where standard triple therapy fails as the first-line treatment. *Helicobacter pylori* (*H. pylori*) eradication rates under 7-day or 14-day regimen are in the range of 63–94%, and no clearly decreasing trend has yet been observed. Studies on the feasibility of extending the treatment duration of BQT to a 14-day regimen have presented different results. The main problem in extending the treatment duration is increase in side effects. The most recent guidelines recommend BQT as first-line treatment in regions with clarithromycin resistance of 15% or higher. Increase in metronidazole resistance is considered a contributing factor for the potential failure of BQT. The risk for decrease in eradication rate is especially high in regions where metronidazole resistance exceeds 30%. Due to the unsatisfactory outcomes of BQT as first- or second-line treatment, which is ascribable to increase in antimicrobial resistance, there is an urgent need for developing a new eradication treatment method.

Keywords

Quadruple therapy · Bismuth · Metronidazole Resistance

45.1 Introduction

Classical quadruple therapy is defined as a 7- or 14-day regimen consisting of proton pump inhibitor (PPI) standard dose b.i.d (twice a day) + bismuth 120 mg q.i.d. (four times a day) + tetracycline 500 mg q.i.d. + metronidazole 500 mg t.i.d (three times a day). Because it contains bismuth, it is usually called bismuth quadruple therapy (BQT) in differentiation with the concomitant non-bismuth quadruple therapy (CNBQT), currently in wide use, in which PPI, clarithromycin, amoxicillin, and metronidazole are simultaneously used.

Bismuth has long been used for the treatment of gastritis and peptic ulcer for the effects of bismuth in protecting the gastric mucosa and curing related diseases. It is known to increase the antimicrobial therapeutic effects and decrease the antimicrobial resistance to *H. pylori* eradication treatment by reducing the colonization density of its strains because it can directly induce their dissolution [1, 2]. An additional advantage of bismuth is that it does not provoke antimicrobial resistance. The classical bismuth-containing quadruple therapy has usually been used as second-line treatment in case

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of failure of clarithromycin-containing first-line triple therapy. More recently, its role as the first-line treatment has become important since the Maastricht VI 2022 Consensus Report [3] recommended BQT as the first-line empiric treatment in regions with clarithromycin resistance exceeding 15%, and the latest version of Korean guidelines recommended BQT to be used as first-line therapy if other first-line therapy options are not available [4].

This work is intended to investigate the treatment achievements of BQT as the first- and second-line treatments for *H. pylori* infection and antimicrobial resistance affecting BQT.

45.2 Efficacy of Bismuth Quadruple Therapy as Second-Line Treatment

In the evidence-based guideline in Korea 2020, BQT for 14 days is recommended as a second-line therapy after failure of standard triple therapy [4]. Korean reports on the eradication rates of BQT as second-line treatment widely vary in 7-day and 14-day regimens, and no clearly decreasing trend has yet been observed [5–12] (Fig. 45.1).

The systematic review conducted for the Korea 2020 guidelines [4] identified 15 randomised controlled trials (RCTs) which compared 31 treatment arms as second-line treatment after failure of first-line PPI-clarithromycin-amoxicillin triple therapy [13–27]. BQT showed pooled eradication rate of 75.5% (95% confidence interval [CI], 71.6–79.1%) (Fig. 45.2). Regarding the treatment duration, because 10-day and 14-day regimens showed similar efficacy, subgroup analysis was conducted to compare 7-day vs. 10- to 14-day BQT. In result, 10- to 14-day therapy showed significantly higher eradication rates (pooled eradication rate 81.6%; 95% CI, 76.9–85.6%) than 7-day therapy (pooled eradication rate 68.4%; 95% CI, 53.0–73.5%) ($p < 0.01$). The systematic review also identified three RCTs comparing 14-day vs. 7-day BQTs. Meta-analysis of these RCTs also showed a significant eradication rate with 14-day regimen than 7-day regimen (risk difference [RD] 0.09; 95% CI, 0.02–0.15; $p < 0.01$).

As the current clinical practice of extending the treatment duration to 14 days carries the risks of increasing side effects and decreasing medication adherence, the issue of medication duration needs to undergo further consideration. Specifically, given the difficulties with BQT medication involving high-dose levels and including two drugs that have to be taken four times a day, coupled with relatively frequent side effects (36.4%) [28], there is an urgent need for developing a new rescue therapy with simplified medication and fewer side effects.

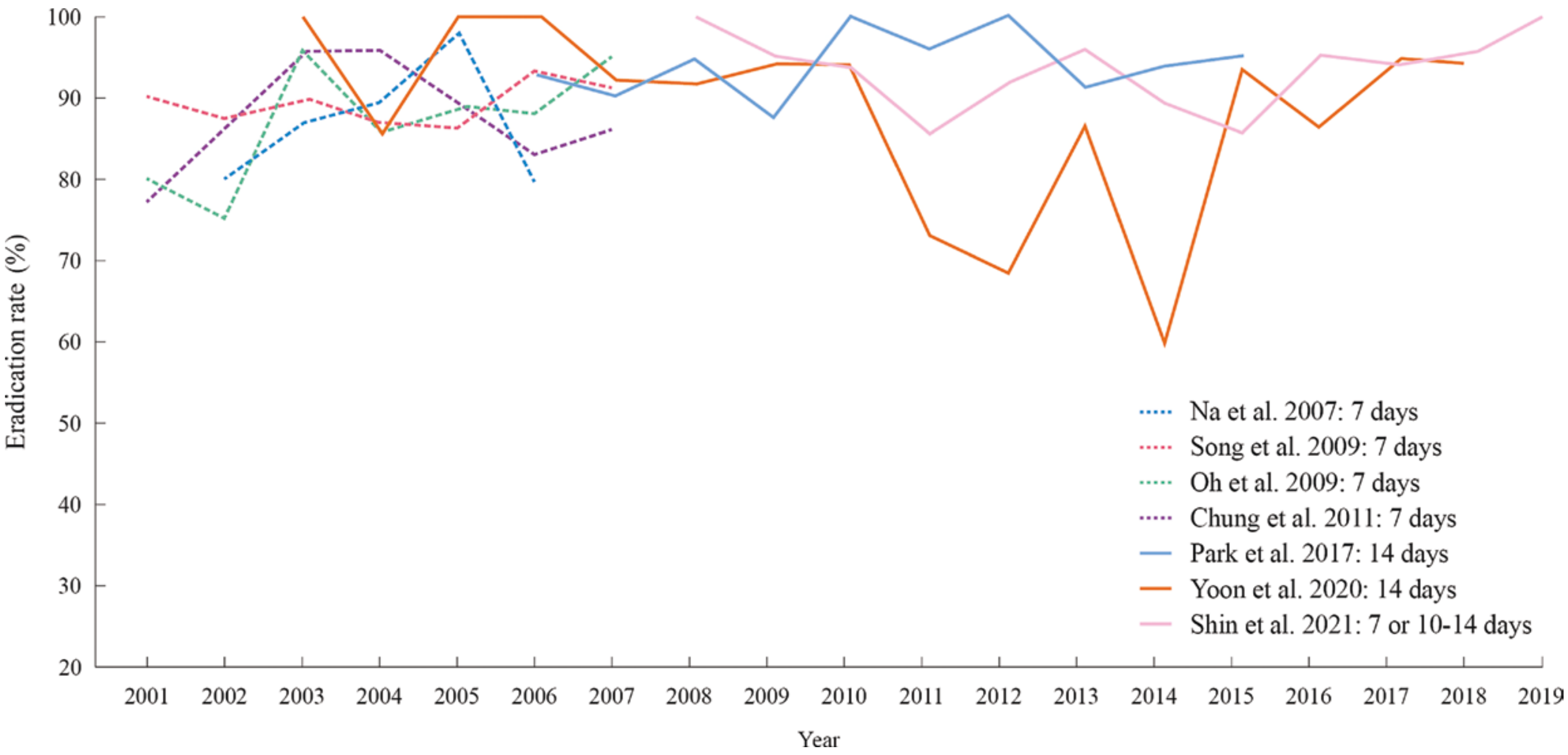
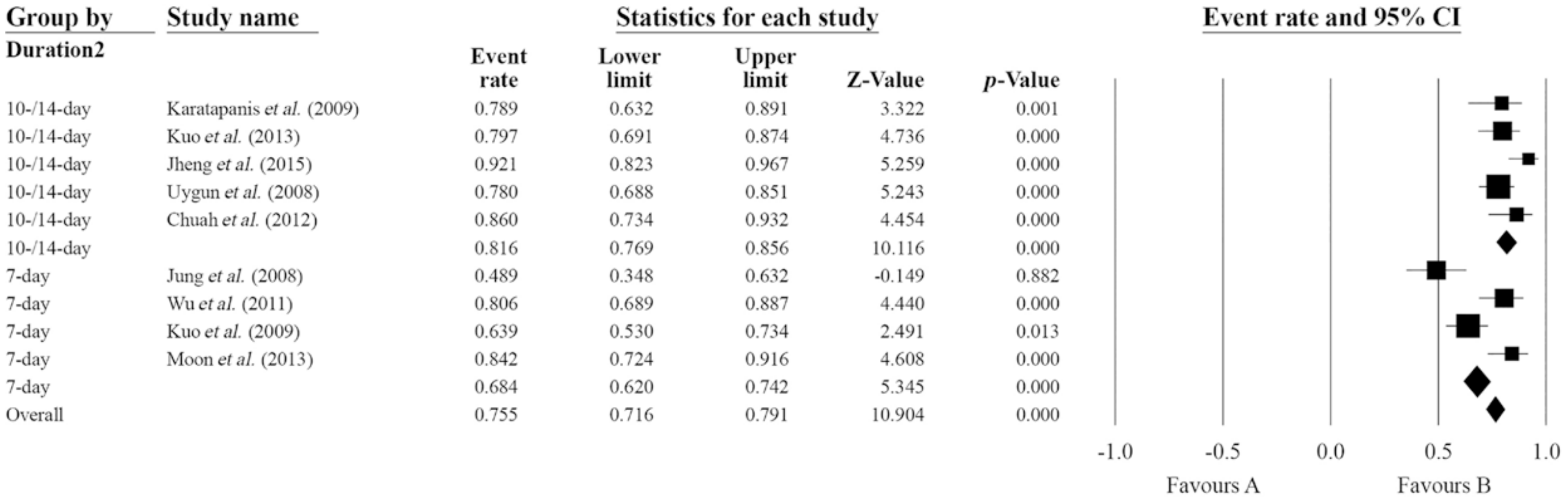


Fig. 45.1 Changes in the per-protocol (PP) eradication rate in South Korea of bismuth quadruple therapy (BQT) used as the second-line rescue therapy. No noticeable decreasing trend in eradication rate is observed. (Modified from Kim and Jung [12])



Group by	Study name	Statistics for each study					Event rate and 95% CI
Duration2		Event rate	Lower limit	Upper limit	Z-Value	*p*-Value	
10-/14-day	Karatapanis *et al.* (2009)	0.789	0.632	0.891	3.322	0.001	
10-/14-day	Kuo *et al.* (2013)	0.797	0.691	0.874	4.736	0.000	
10-/14-day	Jheng *et al.* (2015)	0.921	0.823	0.967	5.259	0.000	
10-/14-day	Uygun *et al.* (2008)	0.780	0.688	0.851	5.243	0.000	
10-/14-day	Chuah *et al.* (2012)	0.860	0.734	0.932	4.454	0.000	
10-/14-day		0.816	0.769	0.856	10.116	0.000	
7-day	Jung *et al.* (2008)	0.489	0.348	0.632	-0.149	0.882	
7-day	Wu *et al.* (2011)	0.806	0.689	0.887	4.440	0.000	
7-day	Kuo *et al.* (2009)	0.639	0.530	0.734	2.491	0.013	
7-day	Moon *et al.* (2013)	0.842	0.724	0.916	4.608	0.000	
7-day		0.684	0.620	0.742	5.345	0.000	
Overall		0.755	0.716	0.791	10.904	0.000	

Fig. 45.2 Meta-analysis of nine studies comparing bismuth quadruple therapy with other regimens after failure of first-line standard triple therapy. The pooled eradication rate of bismuth quadruple therapy as a second-line therapy was 75.5% (95% CI, 71.6–79.1%). (Adapted from Jung et al. [4])

45.3 Efficacy of Bismuth Quadruple Therapy as First-Line Treatment

Theoretically, bismuth-containing agents are easily delivered to the gastric mucosa of patients with dyspepsia with relatively mild inflammatory responses, thus rapidly reaching *H. pylori*, and have low antimicrobial resistance rate, and there have hence been arguments that bismuth-based quadruple therapy should replace the conventional PPI-based triple therapy for *H. pylori*-associated gastritis [29].

Meta-analysis including 9 RCTs from January 2008 to July 2018 investigated the efficacy and safety of BQT for the first-line *H. pylori* eradication [30–38]. Pooled eradication rates of BQT by ITT analysis and PP analysis were 84.5% (95% CI, 74.9–90.9%) and 90.6% (95% CI, 82.8–95.1%), respectively. However, there was no statistically significant difference of ITT eradication rates in 10–14 days BQT compared to 14 days standard triple therapy (relative risk [RR] 1.28; 95% CI, 0.97–1.70) (Fig. 45.3a), 10 days sequential therapy (RR 0.96; 95% CI, 0.83–1.12) (Fig. 45.3b), and 10 days CT (RR 1.01; 95% CI, 0.93–1.10) (Fig. 45.3c). In addition, there was no statistically significant difference of PP eradication rates in 10–14 days bismuth quadruple therapy compared to 14 days standard triple therapy (RR 1.37; 95%, CI, 0.95–1.99), 10 days sequential therapy (RR 0.99; 95% CI, 0.93–1.05), and 10 days concomitant therapy (RR 1.01; 95% CI, 0.95–1.07). In terms of adverse events, BQT was significantly higher than other eradication therapies (RR 1.72; 95% CI, 1.23–2.40). However, considerable heterogeneity was shown among studies ($I^2 = 92\%$) [4].

Another meta-analysis compared standard triple therapy and BQT as first-line treatment and reported the eradication rate of BQT to be much higher than that of standard triple therapy (77.6 vs. 68.9%; RR 1.13; 95% CI, 1.07–1.18) [39]. In this analysis, when 10-day BQT and 7-day standard triple therapies were compared, BQT showed better outcomes, and there were no differences in side effects and patient compliance [39]. However, given the fact that selection and exclusion criteria were different from study to study and individual studies used different PPIs and, above all, the doses of the antibiotics used in BQT slightly varied among the studies, comparing these studies has the disadvantage of great heterogeneity, and particular attention should thus be paid to this issue when interpreting the results. In fact, in the study by Gomollon et al. [40], in which BQT was reported to have eradication rate as low as 68.8%, 250 mg metronidazole was administered three times a day, and Malfertheiner et al. [41] used three-in-one capsule combining bismuth, metronidazole, and tetracycline, which may have contributed to higher medication adherence.

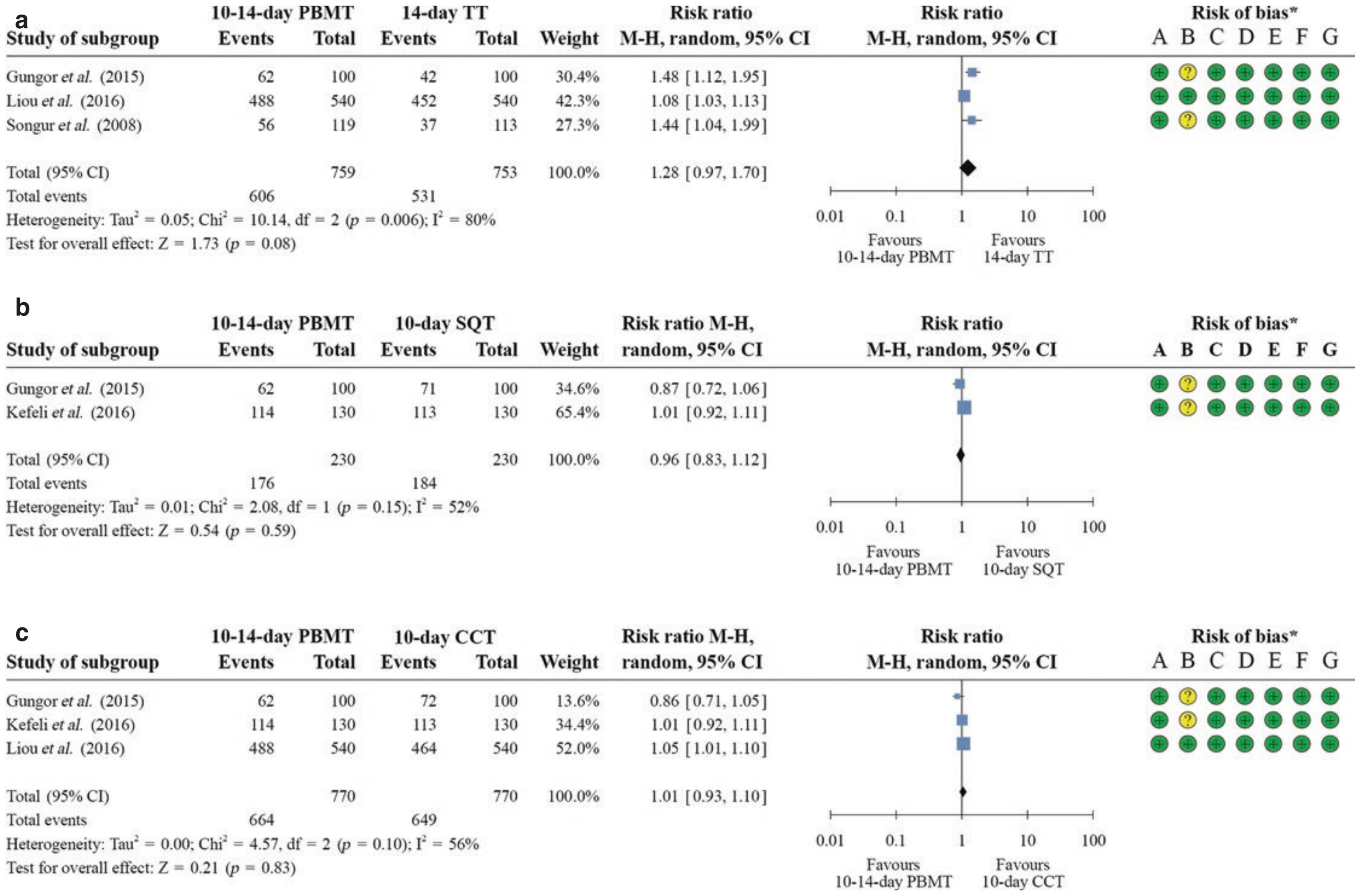


Fig. 45.3 Comparison of the eradication rate of PBMT as first-line therapy in intention-to-treat analysis. (**a**) 10-day/14-day PBMT vs. 14-day TT; (**b**) 10-day/14-day PBMT vs. 10-day SQT; and (**c**) 10-day/14-day PBMT vs. 10-day CCT. *M-H* Mantel-Haenszel test, *CI* confidence interval, *PBMT* bismuth quadruple therapy, *TT* standard triple therapy, *SQT* sequential therapy, *CCT* concomitant therapy. *Risk of bias: *A*, random sequence generation (selection bias); *B*, allocation concealment (selection bias); *C*, blinding of participants and personnel (performance bias); *D*, blinding of outcome assessment (detection bias); *E*, incomplete outcome data (attrition bias); *F*, selective reporting (reporting bias); *G*, other bias. (Adapted from Jung et al. [4])

45.4 Factors Affecting the Bismuth Quadruple Therapy Eradication Rate: Antimicrobial Resistance

Antimicrobial resistance is known to be the main cause of *H. pylori* eradication failure. For standard triple therapy, the key issue is clarithromycin resistance. Antibiotics included in BQT are tetracycline and metronidazole. Because tetracycline resistance is reported to be low globally [42–45], metronidazole resistance may be said to be the main factor affecting BQT's eradiation rate [43]. It is known that metronidazole resistance can be overcome by increasing administration dose or extending medication duration to 10–14 days [46]. For example, a prospective study conducted in China reported that eradication rate over 90% could be achieved with 14-day maximum-dose BQT regimen as first-line empiric treatment (30 mg lansoprazole b.i.d., 220 mg bismuth b.i.d., 500 mg tetracycline q.i.d., and 400 mg metronidazole q.i.d.) without regard to metronidazole resistance [47]. Gerrits et al. [48] reported that metronidazole resistance may decrease the efficacy of metronidazole-containing eradication treatment, but cannot affect the overall outcomes. In a more recent meta-analysis as well [39], eradication rates analyzed from four studies presenting the results related to antimicrobial susceptibility yielded the findings that standard triple therapy eradication rates were 88% and 14.3% in cases with or without clarithromycin resistance, respectively, thus demonstrating the extent to which clarithromycin resistance affects standard triple therapy eradication rate. In contrast, BQT eradication rates with or without metronidazole resistance were not significantly different: 92% without metronidazole resistance and 84.2% with metronidazole resistance. Further research is necessary to investigate the discrepancies between such in vitro metronidazole resistance and actual eradication treatment outcomes. One feasible hypothesis is the difference in oxygen partial pressure. Specifically, metronidazole-resistant *H. pylori* strains can be mistaken as metronidazole-sensitive strains in an environment with low in vitro oxygen partial pressure [16]. Irrespective of such discrepancies, BQT eradication rates are known to fall below 90% in regions with metronidazole resistance rates exceeding 30%, which implies that BQT cannot be a suitable alternative in regions with high antimicrobial resistance [49].

Looking at South Korean reports, the BQT eradication rates of BMT (bismuth + metronidazole + tetracycline) regimen were 100% in the absence of metronidazole resistance and 77.8% when there was metronidazole resistance [50]. The same for BMA (bismuth + metronidazole + amoxicillin) regime were reported to be 91.7 and 70% [51]. While the metronidazole and tetracycline resistance rates of *H. pylori* strains have recently been increasing in South Korea [45, 52], no great changes have been reported on the eradication rate of second-line standard rescue therapy [12]. However, in consideration of the fact that second-line standard rescue therapy involves a smaller number of patients than first-line standard treatment and given the current lack of standard reference concentrations for antimicrobial sensitivity testing as well as various unclarified influential factors, such as in vivo manifestations of antimicrobial sensitivity results, further research with a larger number of patients is necessary [53].

45.5 Conclusions

Although BQT has been applied as second-line therapy and in regions with high clarithromycin resistance as first-line treatment, its treatment performance is not satisfactory. Increasing metronidazole resistance is considered the principal factor for affecting BQT eradication rate, and in South Korea, tetracycline resistance is also relatively high (10–15%), which may exert much influence on further decrease in BQT eradication rate. Therefore, there is an urgent need for intensifying research for developing new first- and second-line therapies capable of replacing BQT.

References

1. Malfertheiner P, Mégraud F, O'Morain C, Hungin AP, Jones R, Axon A, et al. Current concepts in the management of *Helicobacter pylori* infection—the Maastricht 2–2000 Consensus Report. Aliment Pharmacol Ther. 2002;16:167–80.
2. Goodwin CS, Marshall BJ, Blincow ED, Wilson DH, Blackbourn S, Phillips M. Prevention of nitroimidazole resistance in *campylobacter pylori* by coadministration of colloidal bismuth subcitrate: clinical and in vitro studies. J Clin Pathol. 1988;41:207–10.
3. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
4. Jung HK, Kang SJ, Lee YC, Yang HJ, Park SY, Shin CM, et al. Evidence-based guidelines for the treatment of *Helicobacter pylori* infection in Korea 2020. Gut Liver. 2021;15:168–95.
5. Na HS, Hong SJ, Yoon HJ, Maeng JH, Ko BM, Jung IS, et al. Eradication rate of first-line and second-line therapy for *Helicobacter pylori* infection, and reinfection rate after successful eradication. Korean J Gastroenterol. 2007;50:170–5.
6. Song JG, Lee SW, Park JY, Nam SJ, Kim SY, Ahn JH, et al. Trend in the eradication rates of *Helicobacter pylori* infection in the last 11 years. Korean J Med. 2009;76:303–10.
7. Oh J-H, Kim T-H, Cheung D-Y, Park J-M, Kim J-K, Choi S-W, et al. Eradication rates of bismuth-based quadruple therapy as a second-line treatment for *Helicobacter pylori* infection. Korean J Gastrointest Endosc. 2009:131–5.
8. Chung JW, Lee GH, Han JH, Jeong JY, Choi KS, Kim DH, et al. The trends of one-week first-line and second-line eradication therapy for *Helicobacter pylori* infection in Korea. Hepato-Gastroenterology. 2011;58:246–50.
9. Park JS, Park JE, Oh BS, Yoon BW, Kim HK, Lee JW, et al. Trend in the eradication rates of *Helicobacter pylori* infection over the last 10 years in West Gyeonggi-do, Korea: a single center experience. Korean J Gastroenterol. 2017;70:232–8.
10. Yoon K, Kim N, Lee JW, Yoon H, Shin CM, Park YS, et al. Annual eradication rate of bismuth-containing quadruple therapy as second-line treatment for *Helicobacter pylori* infection: a 15-year prospective study at a tertiary hospital in Korea. Helicobacter. 2020;25:e12685.
11. Shin K, Cho MJ, Oh JH, Lim CH. Second-line bismuth-containing quadruple therapy for *Helicobacter pylori* infection: a 12-year study of annual eradication rates. J Clin Med. 2021;10:3273.
12. Kim SY, Jung SW. *Helicobacter pylori* eradication therapy in Korea. Korean J Gastroenterol. 2011;58:67–73.
13. Wu TS, Hsu PI, Kuo CH, Hu HM, Wu IC, Wang SSW, et al. Comparison of 10-day levofloxacin bismuth-based quadruple therapy and levofloxacin-based triple therapy for *Helicobacter pylori*. J Dig Dis. 2017;18:537–42.
14. Chuah SK, Liang CM, Lee CH, Chiou SS, Chiu YC, Hu ML, et al. A randomized control trial comparing 2 levofloxacin-containing second-line therapies for *Helicobacter pylori* eradication. Medicine (Baltimore). 2016;95:e3586.
15. Jheng GH, Wu IC, Shih HY, Wu MC, Kuo FC, Hu HM, et al. Comparison of second-line quadruple therapies with or without bismuth for *Helicobacter pylori* infection. Biomed Res Int. 2015;2015:163960.
16. Moon JY, Kim GH, You HS, Lee BE, Ryu DY, Cheong JH, et al. Levofloxacin, metronidazole, and lansoprazole triple therapy compared to quadruple therapy as a second-line treatment of *Helicobacter pylori* infection in Korea. Gut Liver. 2013;7:406–10.
17. Calhan T, Kahraman R, Sahin A, Senates E, Doganay HL, Kanat E, et al. Efficacy of two levofloxacin-containing second-line therapies for *Helicobacter pylori*: a pilot study. Helicobacter. 2013;18:378–83.
18. Chuah SK, Tai WC, Hsu PI, Wu DC, Wu KL, Kuo CM, et al. The efficacy of second-line anti-*Helicobacter pylori* therapy using an extended 14-day levofloxacin/amoxicillin/proton-pump inhibitor treatment—a pilot study. Helicobacter. 2012;17:374–81.
19. Hu TH, Chuah SK, Hsu PI, Wu DC, Tai WC, Chiu YC, et al. Randomized comparison of two nonbismuth-containing rescue therapies for *Helicobacter pylori*. Am J Med Sci. 2011;342:177–81.
20. Ueki N, Miyake K, Kusunoki M, Shindo T, Kawagoe T, Futagami S, et al. Impact of quadruple regimen of clarithromycin added to metronidazole-containing triple therapy against *Helicobacter pylori* infection following clarithromycin-containing triple-therapy failure. Helicobacter. 2009;14:91–9.
21. Karatapanis S, Skorda L, Georgopoulos S, Papantoniou N, Papastergiou V, Ntoutsikos K, et al. Levofloxacin-based triple therapy versus bismuth-based quadruple therapy as a second line treatment for the eradication of *H. pylori* infection. Ann Gastroenterol. 2009;22:263–7.
22. Uygun A, Ozel AM, Yildiz O, Aslan M, Yesilova Z, Erdil A, et al. Comparison of three different second-line quadruple therapies including bismuth subcitrate in Turkish patients with non-ulcer dyspepsia who failed to eradicate *Helicobacter pylori* with a 14-day standard first-line therapy. J Gastroenterol Hepatol. 2008;23:42–5.
23. Jung HS, Shim KN, Baik SJ, Na YJ, Kang MJ, Jung JM, et al. Efficacy of levofloxacin-based triple therapy as second-line *Helicobacter pylori* eradication. Korean J Gastroenterol. 2008;51:285–90.
24. Kuo CH, Hsu PI, Kuo FC, Wang SS, Hu HM, Liu CJ, et al. Comparison of 10 day bismuth quadruple therapy with high-dose metronidazole or levofloxacin

for second-line *Helicobacter pylori* therapy: a randomized controlled trial. J Antimicrob Chemother. 2013;68:222–8.

25. Wu DC, Hsu PI, Tseng HH, Tsay FW, Lai KH, Kuo CH, et al. *Helicobacter pylori* infection: a randomized, controlled study comparing 2 rescue therapies after failure of standard triple therapies. Medicine (Baltimore). 2011;90:180–5.
26. Chuah SK, Hsu PI, Chang KC, Chiu YC, Wu KL, Chou YP, et al. Randomized comparison of two non-bismuth-containing second-line rescue therapies for *Helicobacter pylori*. Helicobacter. 2012;17:216–23.
27. Kuo CH, Hu HM, Kuo FC, Hsu PI, Chen A, Yu FJ, et al. Efficacy of levofloxacin-based rescue therapy for *Helicobacter pylori* infection after standard triple therapy: a randomized controlled trial. J Antimicrob Chemother. 2009;63:1017–24.
28. Cheon JH, Kim N, Lee DH, Kim JW, Hwang JH, Park YS, et al. Trial of moxifloxacin-containing triple therapy after initial and second-line treatment failures for *Helicobacter pylori* infection. Korean J Gastroenterol. 2005;45:111–7.
29. Jo H-J, Lee D-H, Kang S-J, Kim M-N, Kim S-H, Park J-M, et al. Comparison of the efficacy of bismuth containing PPI-based quadruple therapy with PPI-based triple therapy only as first-line treatment for *Helicobacter pylori* infection. Korean J Gastrointest Endosc. 2008:259–64.
30. Sapmaz F, Kalkan IH, Atasoy P, Basyigit S, Guliter S. A non-inferiority study: modified dual therapy consisting higher doses of rabeprazole is as successful as standard quadruple therapy in eradication of *Helicobacter pylori*. Am J Ther. 2017;24:e393–e8.
31. Kefeli A, Basyigit S, Yeniova AO, Kefeli TT, Aslan M, Tanas O. Comparison of three different regimens against *Helicobacter pylori* as a first-line treatment: a randomized clinical trial. Bosn J Basic Med Sci. 2016;16:52–7.
32. Songur Y, Senol A, Balkarli A, Basturk A, Cerci S. Triple or quadruple tetracycline-based therapies versus standard triple treatment for *Helicobacter pylori* treatment. Am J Med Sci. 2009;338:50–3.
33. Liou JM, Fang YJ, Chen CC, Bair MJ, Chang CY, Lee YC, et al. Concomitant, bismuth quadruple, and 14-day triple therapy in the first-line treatment of *Helicobacter pylori*: a multicentre, open-label, randomised trial. Lancet. 2016;388:2355–65.
34. Gungor G, Baglicakoglu M, Kayacetin E, Biyik M, Ucar R, Goktepe H, et al. Current status of five different regimens for empiric first-line *Helicobacter pylori* erradication in Turkey. Digestion. 2015;92:55–9.
35. Dore MP, Farina V, Cuccu M, Mameli L, Massarelli G, Graham DY. Twice-a-day bismuth-containing quadruple therapy for *Helicobacter pylori* eradication: a randomized trial of 10 and 14 days. Helicobacter. 2011;16:295–300.
36. Gokcan H, Oztas E, Onal IK. Different bismuth-based therapies for eradicating *Helicobacter pylori*: randomized clinical trial of efficacy and safety. Clin Res Hepatol Gastroenterol. 2016;40:124–31.
37. Koksal AS, Onder FO, Torun S, Parlak E, Sayilir A, Tayfur O, et al. Twice a day quadruple therapy for the first-line treatment of *Helicobacter pylori* in an area with a high prevalence of background antibiotic resistance. Acta Gastroenterol Belg. 2013;76:34–7.
38. Tsay FW, Wu DC, Yu HC, Kao SS, Lin KH, Cheng JS, et al. A randomized controlled trial shows that both 14-day hybrid and bismuth quadruple therapies cure most patients with *Helicobacter pylori* infection in populations with moderate antibiotic resistance. Antimicrob Agents Chemother. 2017;61:e00140–17.
39. Venerito M, Krieger T, Ecker T, Leandro G, Malfertheiner P. Meta-analysis of bismuth quadruple therapy versus clarithromycin triple therapy for empiric primary treatment of *Helicobacter pylori* infection. Digestion. 2013;88:33–45.
40. Gomollon F, Valdeperez J, Garuz R, Fuentes J, Barrera F, Malo J, et al. Cost-effectiveness analysis of 2 strategies of *Helicobacter pylori* eradication: results of a prospective and randomized study in primary care. Med Clin (Barc). 2000;115:1–6.
41. Malfertheiner P, Bazzoli F, Delchier JC, Celinski K, Giguere M, Riviere M, et al. *Helicobacter pylori* eradication with a capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline given with omeprazole versus clarithromycin-based triple therapy: a randomised, open-label, non-inferiority, phase 3 trial. Lancet. 2011;377:905–13.
42. Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, et al. *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut. 2013;62:34–42.
43. Graham DY, Fischbach L. *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. Gut. 2010;59:1143–53.
44. Boyanova L, Mitov I. Geographic map and evolution of primary *Helicobacter pylori* resistance to antibacterial agents. Expert Rev Anti-Infect Ther. 2010;8:59–70.
45. Lee JY, Kim N. Future trends of *Helicobacter pylori* eradication therapy in Korea. Korean J Gastroenterol. 2014;63:158–70.
46. Kanizaj TF, Kunac N. *Helicobacter pylori*: future perspectives in therapy reflecting three decades of experience. World J Gastroenterol. 2014;20:699–705.
47. Liang X, Xu X, Zheng Q, Zhang W, Sun Q, Liu W, et al. Efficacy of bismuth-containing quadruple therapies for clarithromycin-, metronidazole-, and fluoroquinolone-resistant *Helicobacter pylori* infections in a prospective study. Clin Gastroenterol Hepatol. 2013;11:802–7.e1.
48. Gerrits MM, van der Wouden EJ, Bax DA, van Zwet AA, van Vliet AH, de Jong A, et al. Role of the rdxA and frxA genes in oxygen-dependent metronidazole resistance of *Helicobacter pylori*. J Med Microbiol. 2004;53:1123–8.
49. Fischbach L, Evans EL. Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for *Helicobacter pylori*. Aliment Pharmacol Ther. 2007;26:343–57.

50. Kim N, Lim C-N, Lim S-H, Lee K-H, Koo M-S, Jung H-C, et al. Establishment of an *Helicobacter pylori*-eradication regimen in consideration of drug resistance, recrudescence and reinfection rate of *H. pylori*. Korean J Med. 1999:279–91.
51. Mun G-H, Hahm J-S, Ryu K-H, Lee O-Y, Han D-S, Yoon B-C, et al. Metronidazole resistance and the eradication of *Helicobacter pylori*. Korean J Gastrointest Endosc. 1998:847–52.
52. Lee JY, Kim N, Kim MS, Choi YJ, Lee JW, Yoon H, et al. Factors affecting first-line triple therapy of *Helicobacter pylori* including CYP2C19 genotype and antibiotic resistance. Dig Dis Sci. 2014;59:1235–43.
53. Kim YK, Kim JS, Kim BW. Recent trends of *Helicobacter pylori* eradication therapy in Korea. Korean J Helicobacter Up Gastrointest Res. 2012;12:219–23.

46

Sequential Therapy

Jung Won Lee

Abstract

Sequential therapy consists of the first half and last half. In the first half, proton pump inhibitor (PPI) and amoxicillin are administrated. Then, PPI, clarithromycin, and antibiotics of nitroimidazole family (metronidazole or tinidazole) are administrated in the last half. This regimen was developed in the theoretical background in which amoxicillin in the first half attenuates the cell wall of *Helicobacter pylori*, inhibits the development of efflux channel, and finally it could overcome the clarithromycin resistance. It is regarded that sequential therapy has higher eradication rate than 'legacy' clarithromycin-containing triple therapy and shows similar adverse events rate with clarithromycin-containing triple therapy. Therefore, sequential therapy is still recommended as an alternative regimen for clarithromycin-containing triple therapy, of which the eradication rate is continuously decreasing all over the world. However, this regimen also has some limitations in that the regimen itself is relatively complicated and it is difficult to find an appropriate second-line rescue treatment when this regimen failed.

Keywords

Helicobacter pylori · Eradication
Sequential therapy

46.1 Introduction

The eradication rate of standard triple therapy for *Helicobacter pylori* (*H. pylori*) infection has been continuously decreasing due to the increase of antibiotic resistance all over the world [1–5]. Above all, the worldwide increase in clarithromycin resistance reduced the efficacy of empirical first-line clarithromycin-containing treatment [2, 6]. When the prevalence of clarithromycin resistance reached at an unacceptable level, the empirical use of clarithromycin-containing triple therapy is no longer recommended [6, 7]. One of several regimens which appeared as an alternative is sequential therapy. Sequential therapy consists of the first half and last half. In the first half, proton pump inhibitor (PPI) and amoxicillin are administrated. Then, PPI, clarithromycin, and antibiotics of nitroimidazole family (metronidazole or tinidazole) are administrated in the last half. Maastricht VI/Florence consensus report published in 2022, European guideline, recommended sequential therapy as an alternative first-line eradication therapy in the areas which show high resistance rate to clarithromycin [8]. However, clarithromycin resistance has increased further worldwide, and questions on the efficacy

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of sequential therapy have been raised. Currently, only Korean and U.S. guidelines still recommend sequential therapy as a possible option for first-line eradication regimen [6, 9].

46.2 Theoretical Background of Sequential Therapy

The following two mechanisms have been suggested to explain why the efficacy of sequential therapy would be superior to that of clarithromycin-containing triple therapy. First, the efficacy of eradication therapy is well known to be inversely proportional to the density of *H. pylori* in the stomach [10]. Dual therapy (PPI + amoxicillin) of the first half of sequential therapy attenuates the density of *H. pylori* and thus it can potentiate the efficacy of subsequent triple therapy (PPI + clarithromycin + nitroimidazole) [11]. Second, one of the main mechanisms for the development of clarithromycin resistance is induction of efflux pump channel at bacterial cell wall which can pump clarithromycin out of cell membrane and can inhibit the binding of clarithromycin to target ribosome [12]. Amoxicillin in the first half of sequential therapy can also weaken the cell wall of *H. pylori* and inhibits the development of efflux pump channel. Through this proposed mechanism, sequential therapy seems to have the potential to overcome clarithromycin resistance. Thus, it could overcome the clarithromycin resistance [11]. However, it is not clear whether the reason that sequential therapy is superior to clarithromycin-containing triple therapy is indeed due to the formerly suggested mechanism or it is simply due to the concomitant administration of antimicrobial agents which also can be used for *H. pylori* eradication.

46.3 Types of Sequential Therapy

The dose of PPI, amoxicillin, and clarithromycin in sequential therapy is the same as that of clarithromycin-containing triple therapy. However, the types and dosage of antibiotics of nitroimidazole family slightly vary with studies. Early studies which were performed in Europe, mainly in Italy, used tinidazole. However, following studies conducted in Asia performed metronidazole-containing treatment. Metronidazole is almost similar to tinidazole except for shorter action time than that of tinidazole. Systematic review demonstrated that there is no significant difference in the eradication efficacy of sequential therapy according to these two antibiotics [13]. The most common dosage for tinidazole and metronidazole is 500 mg b.i.d. (bis in die, twice a day) and 500 mg t.i.d. (ter in die, three times a day), respectively.

The duration of sequential therapy also varies with studies. The most common regimen is 10-day regimen which consists of each 5-day first half and remaining 5-day half. Fourteen or 15-day regimen in which 2 or 3 days were prolonged in each half is the second common regimen [13, 14].

46.4 Outcome of Sequential Therapy

46.4.1 Eradication Rate

A country which reported the outcome of sequential therapy in literature for the first time is Italy. At 2000, the authors reported surprising results that eradication rate in intention-to-treat (ITT) analysis was exceptionally high at 98% [15]. Thereafter, open-label study and double-blind randomized clinical trial which compared sequential therapy with 7-day or 10-day clarithromycin-containing triple therapy consistently reported the supreme efficacy of sequential therapy to clarithromycin-containing triple therapy, and thus sequential therapy has become a popular research topic [16, 17]. Thereafter, many studies regarding sequential therapy were performed, and systematic reviews which summarized these studies also reported that efficacy of sequential therapy was about 91.0–93.5% in ITT analysis and it was superior to that of clarithromycin-containing triple therapy [18–21]. However, these systematic reviews have geographical limitation in that most studies included in the analyses were performed in Italy. Therefore, another systematic review was performed for studies regarding only Asian adults. The authors concluded that sequential therapy is also superior to clarithromycin-containing triple therapy in Asian adults. However, the absolute eradication rate was 81.8% in ITT analysis, and this was approximately 10% lower than previous eradication rates of Western studies [13].

46.4.2 Adverse Events

Because sequential therapy consists of more kinds of antibiotics than clarithromycin-containing triple therapy, there has been a concern that the adverse events of sequential therapy would be more frequently or severely occurred than clarithromycin-containing triple therapy. However, although the rate of adverse events of sequential therapy varied as 8.4–22.6%, majority of systematic reviews concluded that the rate and severity of adverse events of sequential therapy were not significantly higher than those of clarithromycin-containing triple therapy [13, 19, 21].

46.5 Limitations of Sequential Therapy

46.5.1 The Complexity of Regimen

There are many kinds of drugs to be administrated in sequential therapy. In addition, the drugs which are administrated in the first half and the remaining half are different. Therefore, there is a possibility that the compliance of patients is likely to deteriorate [22]. Considering that compliance is the second leading cause for eradication failure, this is a serious problem inherent in sequential therapy itself. One systematic review reported that the compliance of sequential therapy and clarithromycin-containing triple therapy is 97.4% and 96.8%, respectively [18]. One review article reported that when eight studies in which the data regarding the compliance were available were taken together, the rate of the patients who showed compliance higher than 90% was not different between sequential therapy and standard triple therapy (92.6 vs. 94%) [23]. However, considering that compliance in real clinical situation is generally lower than that in the published studies, the possibility that the compliance of sequential therapy is lower than that of clarithromycin-containing triple therapy cannot be ignored.

46.5.2 Rescue Treatment After Failure

Because sequential therapy uses clarithromycin and nitroimidazole which are the most important antimicrobial agents in the empirical first- and second-line *H. pylori* eradication therapy at the same time, it is extremely difficult to determine the next empirical antimicrobial therapy regimen [6, 13, 18]. European guideline suggests levofloxacin-based triple therapy as second-line regimen when first-line sequential therapy failed [7, 8]. However, the eradication rate of levofloxacin-based triple therapy as second-line treatment was approximately 60–70% [6, 7, 24]. Therefore, one study suggested non-quadruple therapy including PPI, bismuth, tetracycline, or levofloxacin as an alternative. However, because the number of the subjects was only 24, the evidence level of that study is not high [25].

46.5.3 Antimicrobial Resistance

To demonstrate that one of the main benefits of sequential therapy compared with clarithromycin-containing triple therapy is to overcome clarithromycin resistance by administration of amoxicillin in the first half, the comparison of eradication rate according to antibiotic resistance of each *H. pylori* strain is required. However, actually there are just a few studies which evaluated antibiotic resistance in sequential therapy. One systematic review reported that the eradication rates of sequential therapy and standard triple therapy for clarithromycin-resistant strain of

H. pylori were 70.7% and 33.8%, respectively [19]. Most studies reported that even though the eradication rate of sequential therapy decreases for clarithromycin-resistant strain of *H. pylori*, the absolute eradication rate of sequential therapy is much higher than that of standard triple therapy [16, 17, 20, 26, 27]. However, because the total number of clarithromycin-resistant strains is small portion, the results should be interpreted with caution. Additionally, recent systematic review by Cochrane concluded that the efficacy of sequential therapy might significantly be reduced by clarithromycin resistance and success rates against clarithromycin-resistant strains were 75% [28]. There is limited number of studies which reported the result of sequential therapy in metronidazole-resistant strain. One meta-analysis reported that the eradication rates of sequential therapy and standard triple therapy for metronidazole-resistant strains were 96.0% and 67.68%, respectively [19].

46.5.4 Insufficient Quality of Clinical Trials

Since 2000s, as resistance to antimicrobial agents has continuously increased worldwide, many studies on sequential therapy have been conducted. However, systematic reviews regarding these concluded that studies in which the number of subjects is more than 100 were rare, the heterogeneity among studies is high, and the quality of study design is relatively low [11, 13, 19–21, 29]. Although we have to interpret the results considering that antibiotic resistance varies with area worldwide and the administration duration of standard triple therapy was long, multi-countries study in Latin America in which the number of subjects was largest among studies regarding sequential therapy suggested that 14-day standard triple therapy was even superior to 10-day sequential therapy [29]. In particular, concerns about the use of inappropriate antibiotics require more attention to sequential therapy, which may include unnecessary antibiotics. Therefore, a well-designed clinical study with sufficient study subject should be followed to establish better eradication strategies for the appropriate use of sequential therapy.

46.6 Conclusions

Sequential therapy is an alternative regimen for clarithromycin-containing triple therapy of which the eradication rate is continuously decreasing all over the world. Taken together many studies that were performed to date, it is thought that sequential therapy has higher eradication rate than standard triple therapy and shows similar adverse events rate with clarithromycin-containing triple therapy. If susceptibility-guided therapy is not available, it is known as effective alternative empirical first-line eradication therapy in many current guidelines as a result of various clinical studies. However, the recent European guideline recommends choosing concomitant therapy rather than sequential therapy, due to the problem of reduced compliance due to the complexity of the regimen. It is also difficult to find an appropriate second-line treatment when this regimen failed. When considering concerns about the use of inappropriate antibiotics, a well-designed high-quality clinical study should be followed to establish better eradication strategies for the appropriate use of sequential therapy.

References

1. Graham DY, Shiotani A. New concepts of resistance in the treatment of *Helicobacter pylori* infections. Nat Clin Pract Gastroenterol Hepatol. 2008;5:321–31.
2. Megraud F, Bruyndonckx R, Coenen S, Wittkop L, Huang TD, Hoebeke M, et al. *Helicobacter pylori* resistance to antibiotics in Europe in 2018 and its relationship to antibiotic consumption in the community. Gut. 2021;70:1815–22.
3. Ho JJC, Navarro M, Sawyer K, Elfanagely Y, Moss SF. *Helicobacter pylori* antibiotic resistance in the United States between 2011 and 2021: a systematic review and meta-analysis. Am J Gastroenterol. 2022;117:1221–30.
4. Lee JW, Kim N, Choi SI, Jang JY, Song CH, Nam RH, et al. Prevalence and trends of multiple antimicrobial resistance of *Helicobacter pylori* in one ter-

tiary hospital for 20 years in Korea. Helicobacter. 2023;28:e12939.

5. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of antibiotic resistance in *Helicobacter pylori*: a systematic review and meta-analysis in World Health Organization Regions. Gastroenterology. 2018;155:1372–82. e17
6. Jung HK, Kang SJ, Lee YC, Yang HJ, Park SY, Shin CM, et al. Evidence based guidelines for the treatment of *Helicobacter pylori* infection in Korea 2020. Korean J Intern Med. 2021;36:807–38.
7. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
8. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of *Helicobacter pylori* infection—the Maastricht IV/ Florence consensus report. Gut. 2012;61:646–64.
9. Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG clinical guideline: treatment of *Helicobacter pylori* infection. Am J Gastroenterol. 2017;112:212–39.
10. Lai YC, Yang JC, Huang SH. Pre-treatment urea breath test results predict the efficacy of *Helicobacter pylori* eradication therapy in patients with active duodenal ulcers. World J Gastroenterol. 2004;10:991–4.
11. Gisbert JP, Calvet X, O'Connor A, Megraud F, O'Morain CA. Sequential therapy for *Helicobacter pylori* eradication: a critical review. J Clin Gastroenterol. 2010;44:313–25.
12. Webber MA, Piddock LJ. The importance of efflux pumps in bacterial antibiotic resistance. J Antimicrob Chemother. 2003;51:9–11.
13. Yoon H, Lee DH, Kim N, Park YS, Shin CM, Kang KK, et al. Meta-analysis: is sequential therapy superior to standard triple therapy for *Helicobacter pylori* infection in Asian adults? J Gastroenterol Hepatol. 2013;28:1801–9.
14. Lee JW, Kim N, Kim JM, Nam RH, Kim JY, Lee JY, et al. A comparison between 15-day sequential, 10-day sequential and proton pump inhibitor-based triple therapy for *Helicobacter pylori* infection in Korea. Scand J Gastroenterol. 2014;49:917–24.
15. Zullo A, Rinaldi V, Winn S, Meddi P, Lionetti R, Hassan C, et al. A new highly effective short-term therapy schedule for *Helicobacter pylori* eradication. Aliment Pharmacol Ther. 2000;14:715–8.
16. Zullo A, Vaira D, Vakil N, Hassan C, Gatta L, Ricci C, et al. High eradication rates of *Helicobacter pylori* with a new sequential treatment. Aliment Pharmacol Ther. 2003;17:719–26.
17. Vaira D, Zullo A, Vakil N, Gatta L, Ricci C, Perna F, et al. Sequential therapy versus standard triple-drug therapy for *Helicobacter pylori* eradication: a randomized trial. Ann Intern Med. 2007;146:556–63.
18. Jafri NS, Hornung CA, Howden CW. Meta-analysis: sequential therapy appears superior to standard therapy for *Helicobacter pylori* infection in patients naive to treatment. Ann Intern Med. 2008;148:923–31.
19. Tong JL, Ran ZH, Shen J, Xiao SD. Sequential therapy vs. standard triple therapies for *Helicobacter pylori* infection: a meta-analysis. J Clin Pharm Ther. 2009;34:41–53.
20. Sirimontaporn N, Thong-Ngam D, Tumwasorn S, Mahachai V. Ten-day sequential therapy of *Helicobacter pylori* infection in Thailand. Am J Gastroenterol. 2010;105:1071–5.
21. Gatta L, Vakil N, Leandro G, Di Mario F, Vaira D. s. Am J Gastroenterol. 2009;104:3069–79; quiz 1080
22. Chung JW, Ha M, Yun SC, Kim JH, Lee JJ, Kim YJ, et al. Meta-analysis: sequential therapy is superior to conventional therapy for *Helicobacter pylori* infection in Korea. Korean J Gastroenterol. 2013;62:267–71.
23. Zullo A, De Francesco V, Hassan C, Morini S, Vaira D. The sequential therapy regimen for *Helicobacter pylori* eradication: a pooled-data analysis. Gut. 2007;56:1353–7.
24. Graham DY. Implications of the paradigm shift in management of *Helicobacter pylori* infections. Ther Adv Gastroenterol. 2023;16:17562848231160858.
25. Hsu PI, Chen WC, Tsay FW, Shih CA, Kao SS, Wang HM, et al. Ten-day quadruple therapy comprising proton-pump inhibitor, bismuth, tetracycline, and levofloxacin achieves a high eradication rate for *Helicobacter pylori* infection after failure of sequential therapy. Helicobacter. 2014;19:74–9.
26. De Francesco V, Margiotta M, Zullo A, Hassan C, Troiani L, Burattini O, et al. Clarithromycin-resistant genotypes and eradication of *Helicobacter pylori*. Ann Intern Med. 2006;144:94–100.
27. Mahachai V, Sirimontaporn N, Tumwasorn S, Thong-Ngam D, Vilaichone RK. Sequential therapy in clarithromycin-sensitive and -resistant *Helicobacter pylori* based on polymerase chain reaction molecular test. J Gastroenterol Hepatol. 2011;26:825–8.
28. Nyssen OP, McNicholl AG, Megraud F, Savarino V, Oderda G, Fallone CA, et al. Sequential versus standard triple first-line therapy for *Helicobacter pylori* eradication. Cochrane Database Syst Rev. 2016;2016:CD009034.
29. Greenberg ER, Anderson GL, Morgan DR, Torres J, Chey WD, Bravo LE, et al. 14-day triple, 5-day concomitant, and 10-day sequential therapies for *Helicobacter pylori* infection in seven Latin American sites: a randomised trial. Lancet. 2011;378:507–14.

47

Concomitant Therapy and Hybrid Therapy

Jung Won Lee

Abstract

Concomitant therapy and hybrid therapy were developed to improve the efficacy of empirical eradication therapy. Concomitant therapy is a regimen in which antimicrobial agent of clarithromycin-containing triple therapy and nitroimidazole are administrated at the same time. This therapy has strength in that it is as superior as sequential therapy to clarithromycin-containing triple therapy in the eradication rate and simplicity than sequential therapy. Therefore, it is currently recommended as a first-line eradication therapy to replace conventional clarithromycin-containing triple therapy in many guidelines in the first-line treatment of *Helicobacter pylori* infection. However, as antimicrobial resistance is gradually increasing worldwide, it is questionable whether the acceptable efficacy of concomitant therapy could be maintained in the future. By adding an antimicrobial agent, there is risk of using an unnecessary antimicrobial agent. This increases the need for antibiotic stewardship and increases the risk of microbiota dysbiosis. In addition, it is not well established how to choose rescue antimicrobial agent when first-line concomitant therapy fails. Hybrid therapy is a regimen in which amoxicillin which is administrated in the first half of sequential therapy is continuously administrated during the last half. Hybrid therapy also demonstrated favorable results along with reverse hybrid therapy, which is being studied recently, but the complexity of the regimen itself is a still unsolved problem.

Keywords

Helicobacter pylori · Eradication
Concomitant therapy · Hybrid therapy

47.1 Introduction

Concomitant therapy is a regimen in which antibiotics of clarithromycin-containing triple therapy and nitroimidazole are administrated at the same time. Therefore, this regimen is also called as bismuth non-containing quadruple therapy. Currently, non-bismuth quadruple therapy is defined as eradication therapy with three antimicrobial agents, amoxicillin, clarithromycin, and metronidazole including concomitant therapy, sequential, and hybrid therapy [1]. The first result of concomitant therapy was reported in 1998 in Germany and Japan; the eradication rate in intention-to-treat (ITT) analysis was high as 96% and 93.3%, respectively [2–4]. Thereafter, studies regarding concomitant therapy were performed all over the world as a better alternative

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than sequential therapy for first-line *Helicobacter pylori* (*H. pylori*) treatment. In addition, there is a regimen called as hybrid therapy which combines sequential therapy and concomitant therapy. Hybrid therapy is a regimen in which amoxicillin which is administrated in the first half of sequential therapy is continuously administrated during the last half. Similar to concomitant therapy, this regimen was also developed to improve the eradication rate of sequential therapy.

47.2 Theoretical Background of Concomitant and Hybrid Therapies

There are some limitations on the sequential therapy behind the emergence and recommendations of concomitant therapy; that is, doubt about whether the high eradication rate of sequential therapy is indeed originated from the administration sequence of antimicrobial agents included in this regimen [4]. If the high eradication rate of sequential therapy is not stemming from the administration sequence of antibiotics and it is rather due to the absolute total number of antibiotics, administration of these four drugs at the same time as carpet bombing would be better than sequential administration in complex, since the former would increase compliance of patient and therefore the eradication rate might be better [5].

The theoretical background for hybrid therapy is that the effect of dual administration of proton pump inhibitor (PPI) and amoxicillin is in proportion to administration duration of these drugs [6]. This hypothesis was tested in Taiwan study in 2011 for the first time [7].

47.3 Outcome of Concomitant and Hybrid Therapies

47.3.1 Eradication Rate

The first systematic review for concomitant therapy which was published in 2009 reported the analysis for 771 patients using nine studies; the eradication rate in ITT and in per-protocol (PP) analysis was 89.7% and 92.9%, respectively [8]. Five studies included in this analysis were randomized controlled trials comparing concomitant therapy and clarithromycin-containing triple therapy. Eradication rate in ITT analysis was 90.8% in concomitant therapy and 79% in clarithromycin-containing triple therapy; that is, concomitant therapy was superior to clarithromycin-containing triple therapy. Systematic review including 1723 patients from 15 studies was published in 2011. The mean eradication rate by ITT analysis was 90% and this was similar to the previous results [9]. In following systematic review which was published in 2012 by the same investigators, the eradication rate by ITT analysis was mean 88% in 2070 patients using 19 studies [10]. When the studies from Latin America and Turkey in which the eradication rate was 74% and 75%, respectively [11, 12], were excluded, the heterogeneity among studies was low and eradication rate by ITT analysis was over 90%.

There are several studies which compared the eradication rate of concomitant therapy and sequential therapy. Studies from Taiwan, China, and Spain which compared 10-day sequential therapy and 10-day concomitant therapy all concluded that there was no significant difference in the eradication rate between the two regimens [13–15]. The multinational study from Latin America compared the eradication rate of 14-day standard triple therapy with 10-day sequential therapy as well as 5-day concomitant therapy; the eradication rate between sequential therapy and concomitant therapy was not different [11]. A study from Taiwan which compared 7-day standard triple therapy, 10-day sequential therapy, and 7-day concomitant therapy also reported that there was no difference in the eradication rate between sequential therapy and concomitant therapy [16].

The duration of concomitant therapy varies from 3 to 14 days. In a systematic review, concomitant therapy demonstrated better eradication efficacy even by short period of administration. However, there was a trend that the eradication rate was higher by prolonged treatment duration (7–10 days) than by short administration (3–5 days) [9]; the eradication rate was 85%, 89%, 93%, and 92% by 3-day, 5-day, 7-day, and 10-day administration, respectively. The optimal duration of concomitant therapy is still under investigation. In Greece, where the prevalence of resistance was high up to 27%, there was no significant difference between 10 and 14 days of treatment (87.9 vs. 87.4%) [17]. However, in a recently published Italian study comparing 10- and 14-day concomitant treatments, the intention-to-treat eradication rate was higher in the 14-day (96.1%) than in the 10-day (80%) [18]. Results from 27 European countries enrolled in Hp-EuReg also demonstrated that the modified intent-to-treat eradication rates for 10- and 14-day treatment regimens were 88.3% and 92.1%, respectively [19]. Therefore, recommended duration of concomitant therapy is a 14-day unless the efficacy of a 10-day regimen is proven locally [1]. Korea is a region known to have a complex problem of resistance to several critical antimicrobial agents for *H. pylori* eradication. Nevertheless, no decrease was observed for the eradication rate of concomitant therapy from 2012 to 2017. Of course, as an empirical therapy, it was shown that the eradication rate of 90% or more was continuously maintained [20].

As opposed to sequential therapy and concomitant therapy which appeared in the literatures already since late 1990s, studies regarding hybrid therapy have been performed since 2010s. The first study which reported the result of hybrid therapy is multicenter study in Taiwan. The authors performed 14-day hybrid therapy in which they administrated PPI and amoxicillin during the first 7 days, and they added clarithromycin and metronidazole to PPI and amoxicillin during the remaining 7 days. The results were surprising; the eradication rate by ITT analysis and PP analysis was 97.4% and 99.1%, respectively [7]. These were approximately 5% improved results compared to 14-day sequential therapy which was performed in the same institutions (eradication by ITT analysis 91.9%, eradication by PP analysis 93.9%) [16]. Hybrid therapy also showed significantly higher eradication rate than sequential therapy in an Iranian study which compared 10-day sequential therapy and 14-day hybrid therapy [21]. However, study from Korea, with high prevalence of antimicrobial resistance, reported that there was no significant difference in the eradication rate between 14-day hybrid therapy and 14-day sequential therapy (eradication by ITT analysis 81.1 vs. 79.8%, $p = 0.821$; eradication by PP analysis 85.9 vs. 82%, $p = 0.489$) [22]. In addition, in a study which compared 14-day hybrid therapy and 14-day concomitant therapy in Italy and Spain, there was no significant difference between the two regimens (eradication rate by ITT analysis 90 vs. 91.7%, $p = 0.35$; eradication by PP analysis 92 vs. 96.1%, $p = 0.07$) [23]. Other than that, multicenter study in Italy compared 5-day concomitant therapy, 10-day sequential therapy, and 14-day hybrid therapy. Although there was no significant difference in the eradication rate, the absolute eradication rate of hybrid therapy was a bit low (eradication rate of concomitant/sequential/hybrid therapy 85.5/91.1/80% by ITT analysis, 91.6/92.1/85.7% by PP analysis) [24]. Another Italian study reported that the eradication rate of 10-day sequential therapy, 14-day concomitant therapy, and 14-day hybrid therapy is similar, and the eradication rate of 5-day concomitant therapy was merely low [25]. The results of the succeeding study in Taiwan which tried shortening of administration duration of 14-day hybrid therapy were recently published. There was no difference in the eradication rate among 10-day, 12-day, and 14-day hybrid therapy (eradication rate by PP analysis 95%, 95.1%, 93.4%, respectively) [15]. Reverse hybrid therapy has recently been proposed, which aims to simplify the sequence of hybrid therapy by first administering four antibiotics and administering PPI and amoxicillin to the remaining half. As a result of reverse hybrid therapy, a recent systematic review involving 4 studies with 1530 participants was reported, with 95.5% (737/772) and 96.2% (701/729) in the ITT and PP analyses, respectively [26].

In summary, because the number of studies regarding hybrid therapy is still small and in studies which compared hybrid therapy with sequential therapy and concomitant therapy the administration duration of each regimen was various, it is hard to draw general conclusions. However, it is thought that the eradication rate of hybrid therapy is generally excellent compared to standard triple therapy.

47.3.2 Adverse Events

According to the first meta-analysis in 2009, the adverse events of concomitant therapy were not different from that of standard triple therapy (27–51% vs. 21–48%); serious adverse events were not reported in any studies [8]. Therefore, although three kinds of antibiotics are administrated in the same time, we could say that concomitant therapy is not different from standard triple therapy in the aspect of adverse events. It is known that the adverse events of hybrid therapy are also not different from those of sequential therapy and concomitant therapy which are the main comparators of hybrid therapy [21–23].

47.4 Limitations of Concomitant Therapy

47.4.1 Rescue Therapy After Eradication Failure

Because antimicrobial agents included in concomitant therapy and hybrid therapy are same as those of sequential therapy, it is equally difficult to select appropriate antimicrobial agents as second-line eradication therapy when these regimens failed as the first line.

47.4.2 Antibiotic Resistance

Several studies suggested that clarithromycin resistance may not significantly affect the efficacy of concomitant therapy [15, 27, 28]. However, because the number of clarithromycin-resistant strains which were included in these studies was very small, the results should be interpreted with caution. The data regarding whether concomitant therapy could overcome metronidazole resistance are also very limited. Although a few studies reported that the eradication rate of concomitant therapy is regardless of metronidazole resistance [2, 29], there is also a study against this assumption [3].

There has been little information in the literature regarding the effect of hybrid therapy on antibiotic-resistant *H. pylori* strains than that of concomitant therapy. Some studies reported that eradication rate of hybrid therapy for clarithromycin and metronidazole-resistant strains was 75–100% and 72.7–100%, respectively [7, 23]. However, the number of resistant strains included in these studies was very small.

In summary, studies regarding whether concomitant therapy and hybrid therapy are effective for antibiotic-resistant *H. pylori* strains are still limited. Therefore, for the present, we can just infer it from the results of studies regarding sequential therapy which uses the same kinds of antibiotics as concomitant therapy and hybrid therapy.

47.5 Conclusions

Concomitant therapy has strength in that it is as effective as sequential therapy to clarithromycin-containing triple therapy in the eradication rate with more straightforward regimen compared to others. Therefore, concomitant therapy is regarded as an effective alternative for conventional clarithromycin-containing triple therapy in many guidelines. However, there is still limitation that the eradication results regarding eradication efficacy for antibiotic-resistant strains are limited and there are limited remaining choices when this regimen fails as the first-line treatment. Hybrid therapy also showed several acceptable results, but did not seem to overcome the complexity of the regimen itself. In order to be continuously recommended as an effective first-line empirical treatment option, more clinical study results should be accumulated.

References

1. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
2. Okada M, Oki K, Shirotani T, Seo M, Okabe N, Maeda K, et al. A new quadruple therapy for the eradication of *Helicobacter pylori*. Effect of pretreatment with omeprazole on the cure rate. J Gastroenterol. 1998;33:640–5.
3. Treiber G, Ammon S, Schneider E, Klotz U. Amoxicillin/metronidazole/omeprazole/clarithromycin: a new, short quadruple therapy for *Helicobacter pylori* eradication. Helicobacter. 1998;3:54–8.
4. Graham DY, Lu H. Is there a role for sequential in sequential anti-*H. pylori* therapy? Gastroenterology. 2006;130:1930–1. author reply 1
5. Zullo A, De Francesco V, Hassan C. Sequential or concomitant therapy for *Helicobacter pylori* eradication? J Clin Gastroenterol. 2010;44:658; author reply -9
6. Graham DY, Lu H, Yamaoka Y. Therapy for *Helicobacter pylori* infection can be improved: sequential therapy and beyond. Drugs. 2008;68:725–36.
7. Hsu PI, Wu DC, Wu JY, Graham DY. Modified sequential *Helicobacter pylori* therapy: proton pump inhibitor and amoxicillin for 14 days with clarithromycin and metronidazole added as a quadruple (hybrid) therapy for the final 7 days. Helicobacter. 2011;16:139–45.
8. Essa AS, Kramer JR, Graham DY, Treiber G. Meta-analysis: four-drug, three-antibiotic, non-bismuth-containing “concomitant therapy” versus triple therapy for *Helicobacter pylori* eradication. Helicobacter. 2009;14:109–18.
9. Gisbert JP, Calvet X. Review article: non-bismuth quadruple (concomitant) therapy for eradication of *Helicobater pylori*. Aliment Pharmacol Ther. 2011;34:604–17.
10. Gisbert JP, Calvet X. Update on non-bismuth quadruple (concomitant) therapy for eradication of *Helicobacter pylori*. Clin Exp Gastroenterol. 2012;5:23–34.
11. Greenberg ER, Anderson GL, Morgan DR, Torres J, Chey WD, Bravo LE, et al. 14-day triple, 5-day concomitant, and 10-day sequential therapies for *Helicobacter pylori* infection in seven Latin American sites: a randomised trial. Lancet. 2011;378:507–14.
12. Toros AB, Ince AT, Kesici B, Saglam M, Polat Z, Uygun A. A new modified concomitant therapy for *Helicobacter pylori* eradication in Turkey. Helicobacter. 2011;16:225–8.
13. Huang YK, Wu MC, Wang SS, Kuo CH, Lee YC, Chang LL, et al. Lansoprazole-based sequential and concomitant therapy for the first-line *Helicobacter pylori* eradication. J Dig Dis. 2012;13:232–8.
14. McNicholl AG, Marin AC, Molina-Infante J, Castro M, Barrio J, Ducons J, et al. Randomised clinical trial comparing sequential and concomitant therapies for *Helicobacter pylori* eradication in routine clinical practice. Gut. 2014;63:244–9.
15. Wu JY, Hsu PI, Wu DC, Graham DY, Wang WM. Feasibility of shortening 14-day hybrid therapy while maintaining an excellent *Helicobacter pylori* eradication rate. Helicobacter. 2014;19:207–13.
16. Hsu PI, Wu DC, Chen WC, Tseng HH, Yu HC, Wang HM, et al. Randomized controlled trial comparing 7-day triple, 10-day sequential, and 7-day concomitant therapies for *Helicobacter pylori* infection. Antimicrob Agents Chemother. 2014;58:5936–42.
17. Apostolopoulos P, Ekmektzoglou K, Georgopoulos S, Chounta E, Theofanopoulou A, Kalantzis C, et al. 10-day versus 14-day quadrupleconcomitant nonbismuth therapy for the treatment of *Helicobacter pylori* infection: results from a randomized prospective study in a high clarithromycin resistance country. J Clin Gastroenterol. 2020;54:522–7.
18. Romano M, Gravina AG, Nardone G, Federico A, Dallio M, Martorano M, et al. Non-bismuth and bismuth quadruple therapies based on previous clarithromycin exposure are as effective and safe in an area of high clarithromycin resistance: a real-life study. Helicobacter. 2020;25:e12694.
19. Nyssen OP, Bordin D, Tepes B, Perez-Aisa A, Vaira D, Caldas M, et al. European Registry on *Helicobacter pylori* management (Hp-EuReg): patterns and trends in first-line empirical eradication prescription and outcomes of 5 years and 21 533 patients. Gut. 2021;70:40–54.
20. Lee BE, Kim JS, Kim BW, Kim JH, Kim JI, Chung JW, et al. Consistency of *Helicobacter pylori* eradication rates of first-line concomitant and sequential therapies in Korea: a nationwide multicenter retrospective study for the last 10 years. Helicobacter. 2021;26:e12780.
21. Sardarian H, Fakheri H, Hosseini V, Taghvaei T, Maleki I, Mokhtare M. Comparison of hybrid and sequential therapies for *Helicobacter pylori* eradication in Iran: a prospective randomized trial. Helicobacter. 2013;18:129–34.
22. Oh DH, Lee DH, Kang KK, Park YS, Shin CM, Kim N, et al. Efficacy of hybrid therapy as first-line regimen for *Helicobacter pylori* infection compared with sequential therapy. J Gastroenterol Hepatol. 2014;29:1171–6.
23. Molina-Infante J, Romano M, Fernandez-Bermejo M, Federico A, Gravina AG, Pozzati L, et al. Optimized nonbismuth quadruple therapies cure most patients with *Helicobacter pylori* infection in populations with high rates of antibiotic resistance. Gastroenterology. 2013;145:121–8. e1
24. Zullo A, Scaccianoce G, De Francesco V, Ruggiero V, D’Ambrosio P, Castorani L, et al. Concomitant,

sequential, and hybrid therapy for *H. pylori* eradication: a pilot study. Clin Res Hepatol Gastroenterol. 2013;37:647–50.

25. De Francesco V, Hassan C, Ridola L, Giorgio F, Ierardi E, Zullo A. Sequential, concomitant and hybrid first-line therapies for *Helicobacter pylori* eradication: a prospective randomized study. J Med Microbiol. 2014;63:748–52.
26. Hu Y, Ouyang Y, Zhu Y, Lu NH. Reverse hybrid therapy for *Helicobacter pylori* eradication: a systematic review and meta-analysis. Helicobacter. 2021;26:e12784.
27. Wu DC, Hsu PI, Wu JY, Opekun AR, Kuo CH, Wu IC, et al. Sequential and concomitant therapy with four drugs is equally effective for eradication of *H. pylori* infection. Clin Gastroenterol Hepatol. 2010;8:36–41.e1
28. Okada M, Nishimura H, Kawashima M, Okabe N, Maeda K, Seo M, et al. A new quadruple therapy for *Helicobacter pylori*: influence of resistant strains on treatment outcome. Aliment Pharmacol Ther. 1999;13:769–74.
29. Neville PM, Everett S, Langworthy H, Tompkins D, Mapstone NP, Axon AT, et al. The optimal antibiotic combination in a 5-day *Helicobacter pylori* eradication regimen. Aliment Pharmacol Ther. 1999;13:497–501.

Tailored Therapy Based on Antibiotic Resistance

48

Yong Hwan Kwon

Abstract

The cure rates of *Helicobacter pylori* (*H. pylori*) eradication therapy using a proton pump inhibitor (PPI) and antimicrobial agents such as amoxicillin (AMX), clarithromycin (CLR), or metronidazole (MTZ) are mainly influenced by bacterial susceptibility to antimicrobial agents. Recently, the eradication rates have gradually decreased because of the increased prevalence of *H. pylori* strains resistant to antimicrobial agents. As the prevalence of antibiotic (e.g., CLR, MTZ, tetracycline, AMX, and fluoroquinolone) resistance was different in the worldwide, local surveillance of antibiotic resistance assessed by *H. pylori* culture and antimicrobial susceptibility testing is warranted to guide clinicians in their choice of therapy. The standard phenotypic testing methods and the molecular testing methods have shown to achieve at least 90% success rate, especially in the era of alarmingly high antibiotic resistance of *H. pylori*. The standard phenotypic methods require the successful culture of live *H. pylori* isolates from gastric biopsies and are indeed time-consuming. However, it is currently the only way to test the susceptibility of *H. pylori* isolates to all the commonly used anti-*H. pylori* antibiotics. Compared to the standard phenotypic methods, the molecular testing method does not require successful *H. pylori* culture and therefore is much more rapid and convenient for clinical use. However, this test is only suitable for detecting CLR and levofloxacin susceptibility profiles in *H. pylori*.

Keywords

Helicobacter pylori · Antibiotic resistance
Eradication · Amoxicillin · Clarithromycin
Metronidazole

48.1 Introduction

Since Marshall and Warren revealed the existence of *H. pylori* in the stomach, numerous clinical trials of eradication of *H. pylori* infection were performed, but the results have remained unsatisfactory for a decade [1, 2]. The effectiveness of the most commonly recommended regimens has declined; eradication ratio of the triple regimen containing clarithromycin (CLR) or metronidazole (MTZ), proton pump inhibitor (PPI), and amoxicillin (AMX) has been decreased to the unacceptable level with a corresponding increase in CLR resistance or MTZ [3–12]. A recent study has revealed that the primary and secondary resistance rates *of H. pylori* to CLR, MTZ, and levofloxacin (LEV) surpassed 15% in almost all WHO regions, suggesting that the standard triple therapy is no longer suitable as first-

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line empiric treatment option unless pretreatment personalized antibiotic susceptibility testing is performed to confirm lack of resistance to these antibiotics [13, 14]. Thus, as with other infectious diseases, *H. pylori* eradication regimen should be based on antibiotic susceptibility results either through phenotypic or molecular methods. Recently, the increasing commercial availability of molecular susceptibility testing, as well as an increasing focus on restriction of therapy to susceptibility-based optimized therapies, makes a worldwide change in the approach to therapy of *H. pylori* infections. From now on, to manage the increase of antibiotic resistance in *H. pylori*, we discussed about the mechanism of antibiotic resistance in *H. pylori* and the efficacy of the tailored therapy for eradication of *H. pylori* based on antibiotic susceptibility.

48.2 The Patient-Specific Therapy: Why Tailored Therapy Is Needed

The cure rates of *H. pylori* infection are influenced by several factors such as antibiotic susceptibility, insufficient inhibition of acid secretion [e.g., cytochrome P4502C19 (*CYP2C19*) genotype, PPI dose, and PPI treatment schedule], bacterial genotypes that reduce virulence (e.g., *cagA*-negative strains and the *vacA* s2 genotype), the environment (e.g., smoking), and compliance [3, 15–17]. The failure of eradication was mainly influenced by an increase in antimicrobial resistance, especially to CLR [1, 10, 11, 17–23]. Numerous studies have shown that antibiotic resistance substantially impairs the efficacy of anti-*H. pylori* therapy [22, 24]. The *H. pylori* infection is associated with geographic areas; the prevalence of resistance rates appears to be partly determined by geographical factors; the prevalence of CLR resistance and MTZ resistance was reported to be from 11.1 to 92.4% and 17.0 to 70.0%, respectively, while AMX resistance was reported 1.0–2.0% worldwide [2, 3, 5, 8, 18, 25–28].

However, the clinical relevance of antibiotic resistance in *H. pylori*-associated diseases is still challenged. Most of all, the critical issue is concerned to resistance of CLR in *H. pylori*. Resistance to CLR is known to be a major cause of failure to eradicate *H. pylori* infection, i.e., point mutations in the *23S rRNA* gene at position 2142 or 2143 (A2143G, A2142G, A2142C of domain V) [10, 23, 29–31]. These resistances seem to substantially reduce the efficacy of all macrolide-containing regimens. The success rates of macrolide-containing dual therapies (with a PPI or bismuth compound) decreased from 68% (61–75%) for macrolide-susceptible strains to 33% (22–45%) for macrolide-resistant strains [21, 22, 32, 33]. The success rates for macrolide-containing triple therapies (with a PPI and AMX, MTZ, or tinidazole) are high in patients with macrolide-susceptible strains (86%, 80–92%), but remains low (25%, 12–38%) for macrolide-resistant strains [18, 21, 22, 34]. Previous reported studies were concerned about the resistance to MTZ; the eradication rate of triple therapy was not affected by the MTZ resistance in *H. pylori* [18, 21, 22, 33, 34]. However, there were contradictive reports that the resistance to MTZ in *H. pylori* does have substantial effect on eradication rate. Resistances to AMX, tetracycline (TC), furazolidone, rifabutin, ciprofloxacin, or other related fluoroquinolones have also been held responsible for therapy failure with these drugs [18, 32, 35–38]; there are not enough data available yet to make an accurate estimate of the effect of these resistances on treatment success (Table 48.1).

Table 48.1 Mode of action, resistance mechanisms, and prevalence of resistance among antimicrobials used for treatment of *H. pylori* infection

Antimicrobial	Commonly used compound	Resistance rates	Mode of action	Mechanism of resistance
Nitroimidazoles	MTZ, tinidazole	20–95%	Reduction of prodrug by nitroreductases leads to formation of nitro anion radicals and imidazole intermediates and subsequent DNA damage	Absence of imidazole reduction caused by reduced or abolished activity of electron transport proteins (e.g., RdxA, FrxA, FdxB)
Macrolides	CLR, erythromycin	0–50%	Binds 23S rRNA ribosomal subunit, resulting in inhibition of protein synthesis	Point mutations in 23S rRNA genes
Penicillins	AMX	0–30%	Binding of beta-lactam antibiotic to penicillin-binding proteins (PBP) inhibits cell division	Decreased binding of AMX to PBP D (tolerance) or PBP1A (resistance caused by point mutation in the *PBP1A* gene) and reduced membrane permeability (resistance)
Tetracyclines	TC	0–10%	Binding to ribosome prevents association with aminoacyl-tRNA and subsequent protein synthesis	Point mutations in *16S rRNA* genes and reduced membrane permeability
Fluoroquinolones	Ciprofloxacin, moxifloxacin, LEV	0–20%	Inhibition of DNA gyrase and topoisomerases, interfering with DNA replication	Point mutations in the DNA gyrase gene, *gyrA*
Rifamycins	Rifabutin	0–2%	Binding to RNA polymerase, resulting in transcription inhibition	Point mutations in the RNA polymerase gene, *rpoB*
Nitrofurans	Furazolidone	0–5%	Reduction of prodrug by nitroreductases leads to formation of nitro anion radicals and subsequent DNA damage	Unknown
Proton pump inhibitor	Omeprazole, lansoprazole, pantoprazole	Not reported	Inhibits the proton motive force of the bacterium and destabilizes its site of colonization in the stomach	Unknown
Bismuth	Bismuth subcitrate, bismuth subsalicylate, ranitidine bismuth citrate	Not reported	Inhibits protein, ATP, and cell membrane synthesis	Unknown

AMX amoxicillin, *CLR* clarithromycin, *MTZ* metronidazole, *TC* tetracycline, *LEV* levofloxacin, *RdxA* oxygen-insensitive NAD(P)H nitroreductase, *FrxA* NAD(P)H flavin nitroreductase, *FdxB* ferredoxin-like protein, *PBP* penicillin-binding proteins

Adapted from Gerrits et al. [39]

48.3 Detection of Antibiotic Resistance in *H. pylori*

Tailored therapy based on antibiotic resistance testing, which includes both phenotypic resistance and genotypic resistance testing (Fig. 48.1), might be a major strategy to overcome eradication failure. Traditional antibiotic susceptibility testing requires the isolation and culture of strains from gastric mucosal specimens. The demanding culture conditions, long culture time, and low culture success rate associated with traditional antibiotic susceptibility testing hamper widespread clinical applications.

48.3.1 Culture-Guided Method for Antibiotic Susceptibility Test in *H. pylori*

Agar dilution is a reliable method to assess antibiotic susceptibility in *H. pylori*. The US National Committee for Clinical Laboratory Standards (NCCLS) has approved this technique as the method of choice to detect resistance to all commonly used antibiotics for anti-*H. pylori* therapy [39] (Table 48.2). In the agar dilution assay, antibiotic susceptibility is assessed by growing *H. pylori* on agar plates containing twofold serial dilutions of the antibiotic [18] (Fig. 48.2). This method can also be done in broth, by the so-called broth microdilution method [40]. Since both methods are time-consuming and not used on a daily basis, the protocols have been simplified; bacteria are grown either in agar or broth containing a critical concentration of the antibiotic necessary to define antibiotic resistance (breakpoint susceptibility testing) [18, 40]. The Clinical and Laboratory Standards Institute

Table 48.2 Proposed clinical antimicrobial breakpoints for *H. pylori*

Antibiotics	Resistant (μg/mL)				
AMX	2	1	0.5	0.25	0.125
Azithromycin	4	2	**1**	0.5	0.25
Ciprofloxacin	4	2	**1**	0.5	0.25
CLR	4	2	**1**	0.5	0.25
MTZ	32	16	**8**	4	2
TC	16	8	**4**	2	1
LEV	2	**1**	0.5	0.25	0.125
Moxifloxacin	2	**1**	0.5	0.25	0.125

AMX amoxicillin, *CLR* clarithromycin, *MTZ* metronidazole, (4*TC* tetracycline, *LEV* levofloxacin
Bold represents the cutoff point of antibiotic-resistant minimal inhibitory concentration (MIC) values

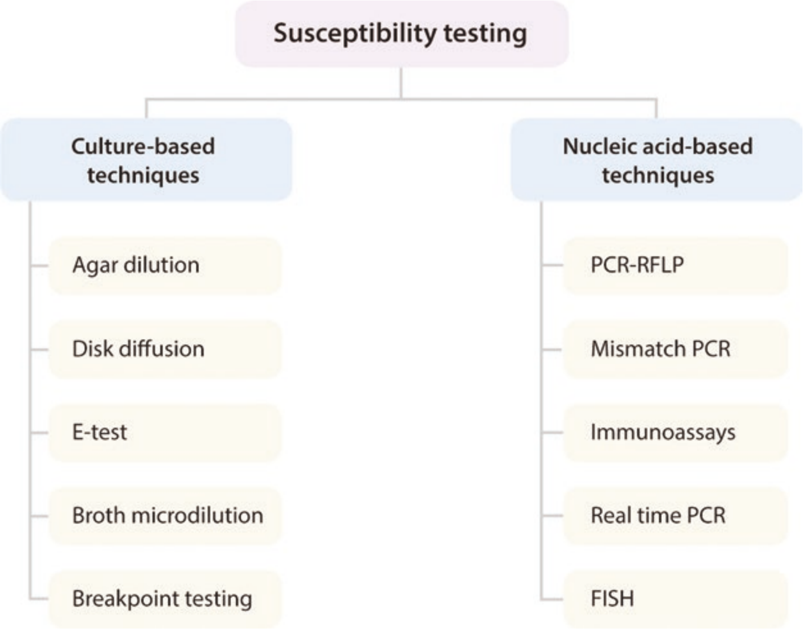

Fig. 48.1 Detection of antibiotic resistance in *H. pylori*. Antibiotic resistance in *H. pylori* can be assessed by culture and nucleic acid-based techniques. *PCR-RFLP* PCR-restriction fragment length polymorphism, *FISH* fluorescent in situ hybridization

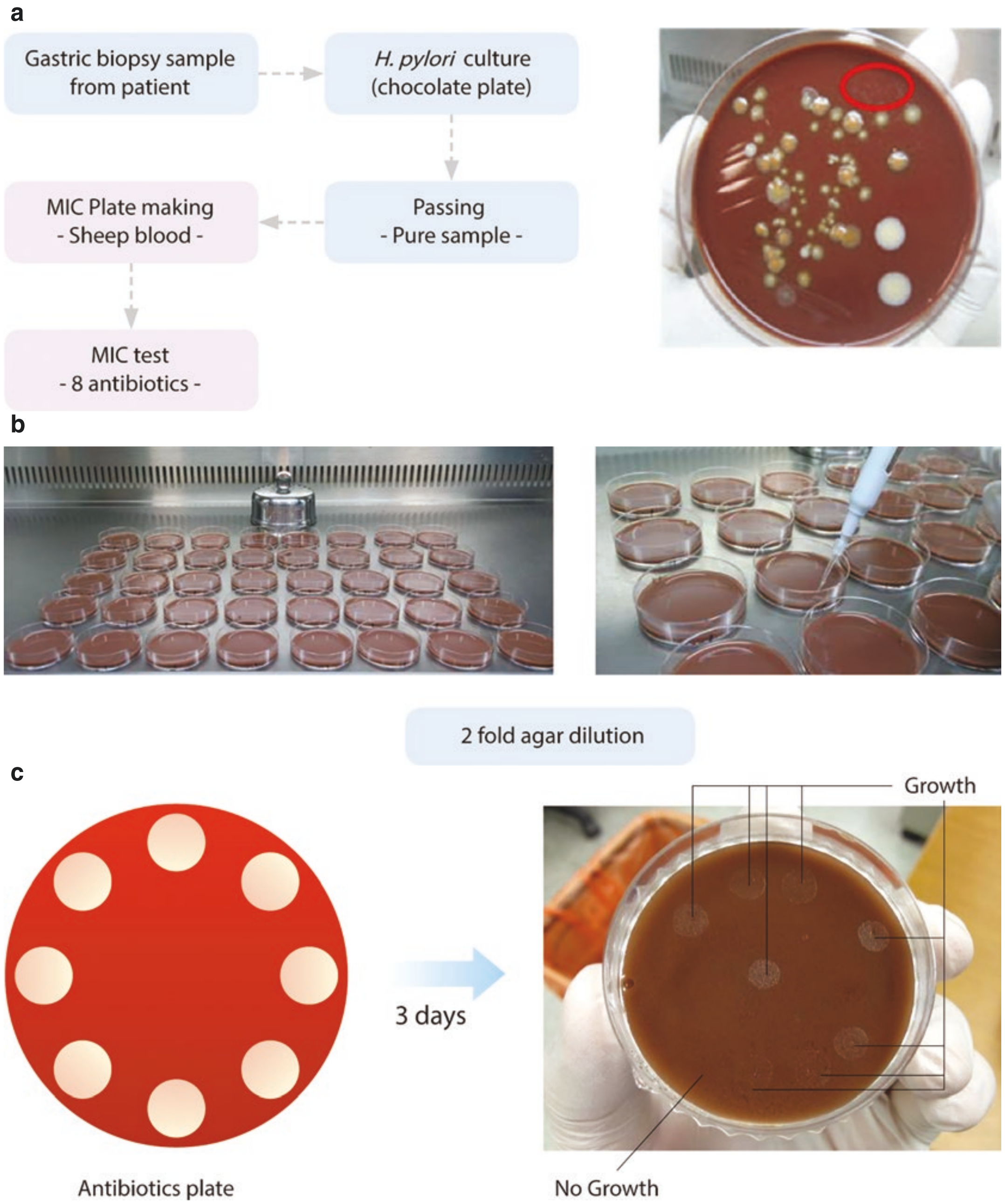
a
Gastric biopsy sample from patient
H. pylori culture (chocolate plate)
Passing - Pure sample -
MIC Plate making - Sheep blood -
MIC test - 8 antibiotics -
b
2 fold agar dilution
c
3 days
Growth
Antibiotics plate
No Growth

Fig. 48.2 *H. pylori* culture and minimal inhibitory concentration (MIC) test. (**a**) For *H. pylori* isolation, gastric biopsy specimens were obtained from the stomach body and antrum during endoscopic examinations. The tissue specimens were cultured on brain-heart infusion agar plates (Difco Laboratories, Detroit, MI., USA) containing 7% horse blood for 3–5 days. Incubation time was extended to 6 or 7 days if the colonies (*red oval*) were too tiny to distinguish. Every *H. pylori* culture was conducted under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2). Cultured bacteria were identified as *H. pylori* by colony morphology, gram staining, and reactions to several enzymatic tests (oxidase, catalase, and urease tests). (**b**) The MIC values of the *H. pylori* isolates to antibiotics were examined using a serial twofold agar dilution method. The bacteria were subcultured on Mueller-Hinton agar supplemented with 5% defibrinated sheep blood for 48 h. The bacterial suspension, adjusted to 1×10^7 CFU, was inoculated directly onto each antibiotic-containing agar dilution plate. (**c**) After 72 h of incubation, the MIC was determined by proposed clinical antimicrobial breakpoints of *H. pylori*

(CLSI) recommends the standardized agar dilution method for susceptibility testing of *H. pylori* and sets the minimal inhibitory concentration (MIC) at ≥1.0 mg/L as CLR resistance breakpoint. However, the clinical MIC breakpoints for other anti-*H. pylori* antibiotics including AMX, MTZ, LEV, TC, and rifabutin have not yet been established [41]. Another cheap and simple method to assess antibiotic susceptibility is the disc diffusion method [18]. In this assay, antibiotic discs are placed on an agar plate with bacteria, and after incubation, antibiotic susceptibility is determined by measuring the inhibition zone [18]. E-test is a commercially available quantitative variant of the disc diffusion method [42, 43]. However, the results of antibiotic resistance identified by agar dilution, disc diffusion, and E-test are not always consistent [18, 40, 42, 43]. MTZ resistance reference, the inhibition zone diameter breakpoint for MTZ resistance obtained from different laboratories, varied from 11 to 20 mm. The inhibition zone diameter breakpoints for CLR resistance varied from 22 to 41 mm and for LEV resistance varied from 9 to 29 mm in different microbiology laboratories around the globe [44–48]. Although the disk diffusion method is the easiest and the most economical method, the current lack of uniform harmonized methodology parameters prevents clinicians from using the disk diffusion method for *H. pylori* susceptibility testing on a routine basis.

48.3.2 Molecular Methods for Antibiotic Susceptibility Test in *H. pylori*

The use of PCR, a fast and inexpensive, culture-free method for susceptibility test, was proven to improve the eradication rate when used to create tailored therapy. They can now be replaced by rapid molecular methods aimed for the genotypic detection of *H. pylori* and its susceptibility to macrolides. They include a standard PCR and other PCR-based methods including PCR-restriction fragment length polymorphism (RFLP), PCR-DNA enzyme immunoassay (DEIA), PCR oligonucleotide ligation assay (OLA), and PCR-line probe assay (LipA) [49, 50]. In recent study, the congruence between the phenotypic antibiotic susceptibility test results by culture and E-test and the molecular susceptibility testing results by whole genome sequencing or next-generation sequencing showed high concordance in CLR and LEV [51, 52]. Real-time PCR assays, representing a powerful advancement of the basic PCR method, have been also developed and are commercially available [50, 53]. These PCR-based culture free methods are accurate in detecting genotypic CLR resistance (sensitivity and specificity reportedly exceeding 80–90% [54]) and offer rapid results as they can be applied directly to biopsy specimens. PCR-RFLP is a PCR-based assay that is commonly used to detect mutational changes in *H. pylori* obtained from biopsy samples or feces [55]. The method is relatively simple and is based on the presence or absence of a restriction site within the amplified DNA fragment. If there is no suitable restriction enzyme available, mutations can be detected using mismatch PCR. Other methods—e.g., PCR-DNA enzyme immunoassay, PCR oligonucleotide ligation assay, preferential homoduplex formation, and PCR-line probe assay—include an additional hybridization step with labelled oligonucleotide probes, specific antibodies, or streptavidin-alkaline phosphatase after the PCR step. It is important that no additional gastric mucosal biopsies are required for PCR testing, as samples originally taken for rapid urease testing have also been utilized [56]. Moreover, freezing specimens for off-site testing may not be necessary, as samples simply stored in a rapid urease test at room temperature for up to 30 days have been successfully used for PCR testing [57]. An alternate approach to DNA amplification and sequencing for the molecular detection of mutations is to use fluorescent in situ hybridization (FISH) [18]. In this method, fluorescent probes specific to mutations associated with resistance are used for the rapid detection of mutations in paraffin-embedded gastric biopsies. FISH is reported to correlate well with results of culture-based susceptibility testing and has the advantage that it may be offered by pathology services using tissue samples sent for histological analysis [58, 59]. Over the past decade, the development of molecular-based *H. pylori* susceptibility testing was rapid. It has been reported that *H. pylori* resistance to MTZ, TC, AMX, and rifabutin is associated to mutations in the *rdxA* and *frxA* genes, the 16S rRNA gene, the *pbp1*, and *rpoB* genes, respectively [52, 60–62]. Based on these antibiotic-related mutations, molecular-based *H. pylori* susceptibility testing methods could be the cornerstone for more appropriate tailored therapy.

48.4 The Efficacy of Tailored Therapy and Guideline for Tailored Therapy Based on Antibiotic Susceptibility Test

According to Maastricht guideline reported from European Helicobacter Study Group, it is not advisable to use standard triple therapy in areas with CLR resistance over 15–20% [63]. It recommends first-line eradication treatment using a bismuth-containing quadruple or non-bismuth containing quadruple regimen in areas with prevalence of CLR or MTZ in areas of high resistance [63]. Unlike most infectious diseases where therapy is guided by knowledge of antibiotic sensitivity profiling of the target organism, or at least by knowledge of strains within the relevant geographic region, *H. pylori* treatment has remained largely empiric. Although antimicrobial susceptibility testing provides the opportunity to tailor therapy for *H. pylori* eradication, susceptibility tests required endoscopy to obtain samples for microbiology examination. Moreover, culturing of *H. pylori* is difficult and several studies have shown that culture success falls below 80% in those who have already failed at least one *H. pylori* eradication therapy [63, 64]. PCR-based approaches have been developed as alternative methods to culture [65]. Early evidence on the benefits of molecular-based methods as a genuine basis for tailoring treatment of *H. pylori* infection has been provided by a few studies. Two Asian studies have provided data on the potential utility of a tailored therapeutic approach based on the molecular detection of *H. pylori* resistance to CLR. In a landmark study, 300 *H. pylori*-positive patients were randomized to either a 1-week standard regimen or to personalized therapy based on both CYP2C19 and CLR susceptibility status assessed by genetic testing [66]. The intention to treat (ITT) eradication rates were significantly higher in the tailored group (96 vs. 70%) without an increase of the final per-patient cost for successful eradication. Tailored treatment using a simple PPI/MTZ regimen successfully eradicated the pathogen in 94.3 vs. 71.4% using empirical standard treatment [30]. In a larger randomized study (1232 participants), CLR was replaced by MTZ when genetic resistance to the former was detected by PCR. This tailored approach was significantly more effective as compared to both CLR- and MTZ-based triple therapies given empirically (eradication rates 91.2 vs. 75.9% and 79.1% respectively; $p < 0.001$ for both comparisons) [23]. In a Korean study (114 patients), ITT eradication rates were 94.7% (95% CI, 88.8–100%) in the culture-guided antimicrobial susceptibility-guided group and 71.9% (95% CI, 60.2–83.5%) in the CLR-based triple therapy group after the initial treatment ($p = 0.002$), whereas the per-protocol (PP) eradication rates were 96.4% (95% CI, 91.5–100%) in the antimicrobial susceptibility-guided group and 73.2% (95% CI, 61.5–84.8%) in the CLR-based triple therapy group ($p = 0.001$) [67].

Many trials were also attempted in *H. pylori* infection by antimicrobial susceptibility in the West (Table. 48.3). However, many studies reported mixed results regarding the efficacy of tailored therapy based on antimicrobial susceptibility testing after *H. pylori* eradication failure. Toracchio et al. [68] reported that eradication rates in the standard group were, respectively, 81 and 75% in 56 patients by PP and ITT analysis, while in the second group they were 98 and 91% in 53 patients ($p < 0.05$) in an open label randomized controlled trial. Cosme et al. [69] showed that, in a population with high CLR resistance, a first-line triple therapy (including omeprazole, CLR, and AMX) achieves better eradication rates if patients are diagnosed by antimicrobial susceptibility testing (88 vs. 49% eradication rate).

Table 48.3 Summary of the culture-guided tailored therapy data

Line of tailored therapy	Author	Type of therapy	Tailored therapy eradication rates per protocol/intention-to-treat analysis
First	Toracchio et al. [68]	Triple	98/91%
	Molina-Infante et al. [70]	Quadruple	92/91%
	Cosme et al. [69]	Triple	88%/NR
	Furuta et al. [66]	Triple	97/96%
	Kawai et al. [30]	Triple	94/94%
Second	Yahav et al. [81]	Triple	86/63%
Third	Gasbarrini et al. [72]	Quadruple	77/52%
	Cammarota et al. [71]	Quadruple	92/91%
	Cammarota et al. [71]	Triple	80/80%
	Gomollón et al. [73]	Quadruple	36–52%/NR
	Vicente et al. [74]	Quadruple	47–74%/NR
Fourth	Fiorini et al. [40]	Triple	89–90%/NR

NR not reported
Adapted from Cammarota et al. [80]

Molina-Infante et al. [70] compared tailored non-bismuth quadruple (concomitant) therapy to either standard triple therapy (in CLR-susceptible *H. pylori* group) or sequential therapy (in CLR-resistant *H. pylori* group). Tailored concomitant therapy was proven to be better than triple therapy and at least as effective as sequential strategy. Cammarota et al. [71] reported excellent results (90% success rate) in 94 patients after treatment with a culture-guided, third-line regimen. In contrast, Gasbarrini et al. [72] showed an eradication rate of 77% or 52% by the PP or ITT analysis by choosing tailored quadruple therapy as a third-line therapy, based on the in vitro sensitivity to the tested antibiotics. Furthermore, according to two trials conducted in Spain [73, 74], the 14-day quadruple therapy administered as a third-line eradication treatment showed eradication rates ranging from 36% to 73.6% depending on the antibiotic regimen used. Taken together, antibiotic resistance rates are rising worldwide and have a significant negative impact on treatment results [8, 12, 26, 75], suggesting that the eradication rate for a third-line therapy could be disappointing when it is administered based on the in vitro sensitivity to the tested antibiotics. The Maastricht VI consensus report stated that “It is reasonable to recommend routinely performing susceptibility tests (molecular or after culture) even before prescribing first-line treatment, with regard to antibiotic stewardship.” However, the generalized use of such a susceptibility-guided strategy in routine clinical practice remains to be established. Several meta-analyses analyzing the efficacy of first-line tailored therapy for *H. pylori* infection found that tailored therapy achieved higher eradication rates than empirical regimens [63, 76]. However, when prescribing only empirical first-line quadruple regimen, no differences in efficacy were found vs. the susceptibility-guided group [63]. Furthermore, those who have failed two or more *H. pylori* therapies have shown similar eradication rates between tailored and empirical therapies [77–79]. Currently, the evidence to support the routine use of susceptibility-guided therapy after *H. pylori* eradication failure is limited and therefore further studies are required to evaluate the benefits of tailored therapy over empirical therapy. Despite these limitations, it is appropriate to recommend that susceptibility tests (culture or PCR) are routinely performed, even before prescribing first-line treatment, in respect to antibiotic stewardship. This provides opportunity to evaluate the prevalence of antibiotic resistance in naïve patients and influence of any such resistance on the effectiveness of up-to-date first-line eradication treatments [63].

48.5 Conclusions

For more than a decade, triple regimens have been the standard of care therapies for *H. pylori* infection. However, in more recent years, worldwide increase in prevalence of antibiotic resistance has accounted for the failure of treatment of *H. pylori* infection. In order to maintain high therapeutic efficacy, regimens with an improved performance against antibiotic-resistant *H. pylori* strains are now recommended as preferred treatment regimens or the fields of genotypic detection of *H. pylori* antimicrobial susceptibility and pharmacogenomics could offer a fascinating new perspective. Accumulating evidence indicates that surveillance of antimicrobial resistance by susceptibility testing is feasible and necessary to inform clinicians in regard to their choice of therapy for management of *H. pylori* infection.

References

1. Graham DY, Fischbach L. *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. Gut. 2010;59:1143–53.
2. Megraud F. *H pylori* antibiotic resistance: prevalence, importance, and advances in testing. Gut. 2004;53:1374–84.
3. Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, et al. *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut. 2013;62:34–42.
4. Rodgers C, van Zanten SV. A meta-analysis of the success rate of *Helicobacter pylori* therapy in Canada. Can J Gastroenterol. 2007;21:295–300.
5. Selgrad M, Meissle J, Bornschein J, Kandulski A, Langner C, Varbanova M, et al. Antibiotic susceptibility of *Helicobacter pylori* in Central Germany and its relationship with the number of eradication therapies. Eur J Gastroenterol Hepatol. 2013;25:1257–60.
6. Wu W, Yang Y, Sun G. Recent insights into antibiotic resistance in *Helicobacter pylori* eradication. Gastroenterol Res Pract. 2012;2012:723183.
7. Karamanolis GP, Daikos GL, Xouris D, Goukos D, Delladetsima I, Ladas SD. The evolution of *Helicobacter pylori* antibiotics resistance over 10 years in Greece. Digestion. 2014;90:229–31.
8. Horiki N, Omata F, Uemura M, Suzuki S, Ishii N, Iizuka Y, et al. Annual change of primary resistance to clarithromycin among *Helicobacter pylori* isolates from 1996 through 2008 in Japan. Helicobacter. 2009;14:86–90.
9. Gao W, Cheng H, Hu F, Li J, Wang L, Yang G, et al. The evolution of *Helicobacter pylori* antibiotics resistance over 10 years in Beijing, China. Helicobacter. 2010;15:460–6.
10. De Francesco V, Giorgio F, Ierardi E, Zotti M, Neri M, Milano A, et al. Primary clarithromycin resistance in *Helicobacter pylori*: the multicentric Italian clarithromycin resistance observational (MICRO) study. J Gastrointestin Liver Dis. 2011;20:235–9.
11. Fischbach L, Evans EL. Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for *Helicobacter pylori*. Aliment Pharmacol Ther. 2007;26:343–57.
12. Lee JW, Kim N, Kim JM, Nam RH, Chang H, Kim JY, et al. Prevalence of primary and secondary antimicrobial resistance of *Helicobacter pylori* in Korea from 2003 through 2012. Helicobacter. 2013;18:206–14.
13. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of antibiotic resistance in *Helicobacter pylori*: a systematic review and meta-analysis in World Health Organization regions. Gastroenterology. 2018;155:1372–82.e17.
14. Yang T, Hu R, Tang X, Shen Y, Tay A, Pi X, et al. Susceptibility-guided bismuth quadruple therapies for resistant *Helicobacter pylori* infections. Precis Clin Med. 2020;3:127–35.
15. Megraud F. Helicobacter pylori and antibiotic resistance. Gut. 2007;56:1502.
16. Yang JC, Lin CJ. CYP2C19 genotypes in the pharmacokinetics/pharmacodynamics of proton pump inhibitor-based therapy of *Helicobacter pylori* infection. Expert Opin Drug Metab Toxicol. 2010;6:29–41.
17. Georgopoulos SD, Ladas SD, Karatapanis S, Mentis A, Spiliadi C, Artikis V, et al. Factors that may affect treatment outcome of triple *Helicobacter pylori* eradication therapy with omeprazole, amoxicillin, and clarithromycin. Dig Dis Sci. 2000;45:63–7.
18. Gerrits MM, van Vliet AH, Kuipers EJ, Kusters JG. *Helicobacter pylori* and antimicrobial resistance: molecular mechanisms and clinical implications. Lancet Infect Dis. 2006;6:699–709.
19. Fontana C, Favaro M, Pietroiusti A, Pistoia ES, Galante A, Favalli C. Detection of clarithromycin-resistant *Helicobacter pylori* in stool samples. J Clin Microbiol. 2003;41:3636–40.
20. De Francesco V, Giorgio F, Hassan C, Manes G, Vannella L, Panella C, et al. Worldwide *H. pylori* antibiotic resistance: a systematic review. J Gastrointestin Liver Dis. 2010;19:409–14.
21. Alarcón T, Domingo D, López-Brea M. Antibiotic resistance problems with *Helicobacter pylori*. Int J Antimicrob Agents. 1999;12:19–26.
22. Houben MH, van de Beek D, Hensen EF, de Craen AJ, Rauws EA, Tytgat GN. A systematic review of *Helicobacter pylori* eradication therapy—the impact of antimicrobial resistance on eradication rates. Aliment Pharmacol Ther. 1999;13:1047–55.
23. Lee HJ, Kim JI, Cheung DY, Kim TH, Jun EJ, Oh JH, et al. Eradication of *Helicobacter pylori* according to 23S ribosomal RNA point mutations associ-

ated with clarithromycin resistance. J Infect Dis. 2013;208:1123–30.

24. Kanizaj TF, Kunac N. *Helicobacter pylori*: future perspectives in therapy reflecting three decades of experience. World J Gastroenterol. 2014;20:699–705.
25. Magalhães Queiroz DM, Luzza F. Epidemiology of *Helicobacter pylori* infection. Helicobacter. 2006;11(Suppl 1):1–5.
26. McMahon BJ, Hennessy TW, Bensler JM, Bruden DL, Parkinson AJ, Morris JM, et al. The relationship among previous antimicrobial use, antimicrobial resistance, and treatment outcomes for *Helicobacter pylori* infections. Ann Intern Med. 2003;139:463–9.
27. Oleastro M, Cabral J, Ramalho PM, Lemos PS, Paixão E, Benoliel J, et al. Primary antibiotic resistance of *Helicobacter pylori* strains isolated from Portuguese children: a prospective multicentre study over a 10 year period. J Antimicrob Chemother. 2011;66:2308–11.
28. Su P, Li Y, Li H, Zhang J, Lin L, Wang Q, et al. Antibiotic resistance of *Helicobacter pylori* isolated in the southeast coastal region of China. Helicobacter. 2013;18:274–9.
29. Alarcón T, Domingo D, Prieto N, López-Brea M. PCR using 3′-mismatched primers to detect A2142C mutation in 23S rRNA conferring resistance to clarithromycin in *Helicobacter pylori* clinical isolates. J Clin Microbiol. 2000;38:923–5.
30. Kawai T, Yamagishi T, Yagi K, Kataoka M, Kawakami K, Sofuni A, et al. Tailored eradication therapy based on fecal *Helicobacter pylori* clarithromycin sensitivities. J Gastroenterol Hepatol. 2008;23(Suppl 2):171–4.
31. Maeda S, Yoshida H, Ogura K, Kanai F, Shiratori Y, Omata M. *Helicobacter pylori* specific nested PCR assay for the detection of 23S rRNA mutation associated with clarithromycin resistance. Gut. 1998;43:317–21.
32. Mégraud F, Doermann HP. Clinical relevance of resistant strains of *Helicobacter pylori*: a review of current data. Gut. 1998;43(Suppl 1):61–5.
33. Dore MP, Leandro G, Realdi G, Sepulveda AR, Graham DY. Effect of pretreatment antibiotic resistance to metronidazole and clarithromycin on outcome of *Helicobacter pylori* therapy: a meta-analytical approach. Dig Dis Sci. 2000;45:68–76.
34. van der Wouden EJ, Thijs JC, van Zwet AA, Sluiter WJ, Kleibeuker JH. The influence of in vitro nitroimidazole resistance on the efficacy of nitroimidazole-containing anti-*Helicobacter pylori* regimens: a meta-analysis. Am J Gastroenterol. 1999;94:1751–9.
35. Ecclissato C, Marchioretto MA, Mendonça S, Godoy AP, Guersoni RA, Deguer M, et al. Increased primary resistance to recommended antibiotics negatively affects *Helicobacter pylori* eradication. Helicobacter. 2002;7:53–9.
36. Heep M, Kist M, Strobel S, Beck D, Lehn N. Secondary resistance among 554 isolates of *Helicobacter pylor*i after failure of therapy. Eur J Clin Microbiol Infect Dis. 2000;19:538–41.
37. Xia H, Keane CT, Beattie S, O'Morain CA. Standardization of disk diffusion test and its clinical significance for susceptibility testing of metronidazole against *Helicobacter pylori*. Antimicrob Agents Chemother. 1994;38:2357–61.
38. Dore MP, Graham DY, Sepulveda AR. Different penicillin-binding protein profiles in amoxicillin-resistant *Helicobacter pylori*. Helicobacter. 1999;4:154–61.
39. Hsueh PR, Ko WC, Wu JJ, Lu JJ, Wang FD, Wu HY, et al. Consensus statement on the adherence to clinical and laboratory standards institute (CLSI) antimicrobial susceptibility testing guidelines (CLSI-2010 and CLSI-2010-update) for *Enterobacteriaceae* in clinical microbiology laboratories in Taiwan. J Microbiol Immunol Infect. 2010;43:452–5.
40. Fiorini G, Vakil N, Zullo A, Saracino IM, Castelli V, Ricci C, et al. Culture-based selection therapy for patients who did not respond to previous treatment for *Helicobacter pylori* infection. Clin Gastroenterol Hepatol. 2013;11:507–10.
41. CLSI, editor. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria, CLSI guideline M45. 3rd ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
42. Megraud F, Lehn N, Lind T, Bayerdörffer E, O'Morain C, Spiller R, et al. Antimicrobial susceptibility testing of *Helicobacter pylori* in a large multicenter trial: the MACH 2 study. Antimicrob Agents Chemother. 1999;43:2747–52.
43. van der Wouden EJ, de Jong A, Thijs JC, Kleibeuker JH, van Zwet AA. Subpopulations of *Helicobacter pylori* are responsible for discrepancies in the outcome of nitroimidazole susceptibility testing. Antimicrob Agents Chemother. 1999;43:1484–6.
44. Miftahussurur M, Fauzia KA, Nusi IA, Setiawan PB, Syam AF, Waskito LA, et al. E-test versus agar dilution for antibiotic susceptibility testing of *Helicobacter pylori*: a comparison study. BMC Res Notes. 2020;13:22.
45. Tang X, Shen Y, Hu R, Yang T, Benghezal M, Li H, et al. Re-assessment of the disk diffusion technique for routine antimicrobial susceptibility testing for *Helicobacter pylori*. Helicobacter. 2020;25:e12703.
46. Yu C, Li L, Chen W, Jiao Y, Yang N, Yang E, et al. Levofloxacin susceptibility testing for *Helicobacter pylori* in China: comparison of E-test and disk diffusion method. Helicobacter. 2011;16:119–23.
47. Knezevic P, Aleksic Sabo V, Simin N, Lesjak M, Mimica-Dukic N. A colorimetric broth microdilution method for assessment of *Helicobacter pylori* sensitivity to antimicrobial agents. J Pharm Biomed Anal. 2018;152:271–8.
48. Li H, Shen Y, Song X, Tang X, Hu R, Marshall BJ, et al. Need for standardization and harmonization of *Helicobacter pylori* antimicrobial susceptibility testing. Helicobacter. 2022;27:e12873.
49. Lehours P, Siffré E, Mégraud F. DPO multiplex PCR as an alternative to culture and susceptibility testing

to detect *Helicobacter pylori* and its resistance to clarithromycin. BMC Gastroenterol. 2011;11:112.

50. Mégraud F, Lehours P. *Helicobacter pylori* detection and antimicrobial susceptibility testing. Clin Microbiol Rev. 2007;20:280–322.
51. Tuan VP, Narith D, Tshibangu-Kabamba E, Dung HDQ, Viet PT, Sokomoth S, et al. A next-generation sequencing-based approach to identify genetic determinants of antibiotic resistance in Cambodian *Helicobacter pylori* clinical isolates. J Clin Med. 2019;8:858.
52. Lauener FN, Imkamp F, Lehours P, Buissonnière A, Benejat L, Zbinden R, et al. Genetic determinants and prediction of antibiotic resistance phenotypes in *Helicobacter pylori*. J Clin Med. 2019;8:53.
53. Papastergiou V, Georgopoulos SD, Karatapanis S. Current and future insights in *H. pylori* eradication regimens: the need of tailoring therapy. Curr Pharm Des. 2014;20:4521–32.
54. Rimbara E, Fischbach LA, Graham DY. Optimal therapy for *Helicobacter pylori* infections. Nat Rev Gastroenterol Hepatol. 2011;8:79–88.
55. Klesiewicz K, Nowak P, Karczewska E, Skiba I, Wojtas-Bonior I, Sito E, et al. PCR-RFLP detection of point mutations A2143G and A2142G in 23S rRNA gene conferring resistance to clarithromycin in *Helicobacter pylori* strains. Acta Biochim Pol. 2014;61:311–5.
56. Furuta T, Sagehashi Y, Shirai N, Sugimoto M, Nakamura A, Kodaira M, et al. Influence of CYP2C19 polymorphism and *Helicobacter pylori* genotype determined from gastric tissue samples on response to triple therapy for *H pylori* infection. Clin Gastroenterol Hepatol. 2005;3:564–73.
57. Li Y, Rimbara E, Thirumurthi S, Trespalacios A, Reddy R, Sabounchi S, et al. Detection of clarithromycin resistance in *Helicobacter pylori* following noncryogenic storage of rapid urease tests for 30 days. J Dig Dis. 2012;13:54–9.
58. Yilmaz O, Demiray E, Tümer S, Altungöz O, Yörükoglu K, Soytürk M, et al. Detection of *Helicobacter pylori* and determination of clarithromycin susceptibility using formalin-fixed, paraffin-embedded gastric biopsy specimens by fluorescence in situ hybridization. Helicobacter. 2007;12:136–41.
59. Morris JM, Reasonover AL, Bruce MG, Bruden DL, McMahon BJ, Sacco FD, et al. Evaluation of seaFAST, a rapid fluorescent in situ hybridization test, for detection of *Helicobacter pylori* and resistance to clarithromycin in paraffin-embedded biopsy sections. J Clin Microbiol. 2005;43:3494–6.
60. Okamoto T, Yoshiyama H, Nakazawa T, Park ID, Chang MW, Yanai H, et al. A change in PBP1 is involved in amoxicillin resistance of clinical isolates of *Helicobacter pylori*. J Antimicrob Chemother. 2002;50:849–56.
61. Qureshi NN, Gallaher B, Schiller NL. Evolution of amoxicillin resistance of *Helicobacter pylori* in vitro: characterization of resistance mechanisms. Microb Drug Resist. 2014;20:509–16.
62. Jenks PJ, Edwards DI. Metronidazole resistance in *Helicobacter pylori*. Int J Antimicrob Agents. 2002;19:1–7.
63. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62. https://doi.org/10.1136/gutjnl-2022-327745.
64. Gisbert JP. Empirical or susceptibility-guided treatment for *Helicobacter pylori* infection? A comprehensive review. Ther Adv Gastroenterol. 2020;13:1756284820968736.
65. Ierardi E, Giorgio F, Iannone A, Losurdo G, Principi M, Barone M, et al. Noninvasive molecular analysis of *Helicobacter pylori*: is it time for tailored first-line therapy? World J Gastroenterol. 2017;23:2453–8.
66. Furuta T, Shirai N, Kodaira M, Sugimoto M, Nogaki A, Kuriyama S, et al. Pharmacogenomics-based tailored versus standard therapeutic regimen for eradication of *H. pylori*. Clin Pharmacol Ther. 2007;81:521–8.
67. Park CS, Lee SM, Park CH, Koh HR, Jun CH, Park SY, et al. Pretreatment antimicrobial susceptibility-guided vs. clarithromycin-based triple therapy for *Helicobacter pylori* eradication in a region with high rates of multiple drug resistance. Am J Gastroenterol. 2014;109:1595–602.
68. Toracchio S, Cellini L, Di Campli E, Cappello G, Malatesta MG, Ferri A, et al. Role of antimicrobial susceptibility testing on efficacy of triple therapy in *Helicobacter pylori* eradication. Aliment Pharmacol Ther. 2000;14:1639–43.
69. Cosme A, Montes M, Martos M, Gil I, Mendarte U, Salicio Y, et al. Usefulness of antimicrobial susceptibility in the eradication of *Helicobacter pylori*. Clin Microbiol Infect. 2013;19:379–83.
70. Molina-Infante J, Pazos-Pacheco C, Vinagre-Rodriguez G, Perez-Gallardo B, Dueñas-Sadornil C, Hernandez-Alonso M, et al. Nonbismuth quadruple (concomitant) therapy: empirical and tailored efficacy versus standard triple therapy for clarithromycin-susceptible *Helicobacter pylori* and versus sequential therapy for clarithromycin-resistant strains. Helicobacter. 2012;17:269–76.
71. Cammarota G, Martino A, Pirozzi G, Cianci R, Branca G, Nista EC, et al. High efficacy of 1-week doxycycline- and amoxicillin-based quadruple regimen in a culture-guided, third-line treatment approach for *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2004;19:789–95.
72. Gasbarrini A, Ojetti V, Armuzzi A, Branca G, Canducci F, Torre ES, et al. Efficacy of a multistep strategy for *Helicobacter pylori* eradication. Aliment Pharmacol Ther. 2000;14:79–83.
73. Gomollon F, Sicilia B, Ducóns JA, Sierra E, Revillo MJ, Ferrero M. Third line treatment for *Helicobacter pylori*: a prospective, culture-guided study in peptic ulcer patients. Aliment Pharmacol Ther. 2000;14:1335–8.
74. Vicente R, Sicilia B, Gallego S, Revillo MJ, Ducons J, Gomollón F. *Helicobacter pylori* eradication in

patients with peptic ulcer after two treatment failures: a prospective culture-guided study. Gastroenterol Hepatol. 2002;25:438–42.

75. Graham DY, Dore MP. *Helicobacter pylori* therapy demystified. Helicobacter. 2011;16:343–5.
76. Gingold-Belfer R, Niv Y, Schmilovitz-Weiss H, Levi Z, Boltin D. Susceptibility-guided versus empirical treatment for *Helicobacter pylori* infection: a systematic review and meta-analysis. J Gastroenterol Hepatol. 2021;36:2649–58.
77. Liou JM, Chen CC, Chang CY, Chen MJ, Fang YJ, Lee JH, et al. Efficacy of genotypic resistance-guided sequential therapy in the third-line treatment of refractory *Helicobacter pylori* infection: a multicentre clinical trial. J Antimicrob Chemother. 2013;68:450–6.
78. Huang HT, Wang HM, Yang SC, Tai WC, Liang CM, Wu KL, et al. Efficacy of a 14-day quadruple-therapy regimen for third-line *Helicobacter pylori* eradication. Infect Drug Resist. 2018;11:2073–80.
79. Puig I, López-Góngora S, Calvet X, Villoria A, Baylina M, Sanchez-Delgado J, et al. Systematic review: third-line susceptibility-guided treatment for *Helicobacter pylori* infection. Ther Adv Gastroenterol. 2016;9:437–48.
80. Cammarota G, Ianiro G, Bibbò S, Di Rienzo TA, Masucci L, Sanguinetti M, et al. Culture-guided treatment approach for *Helicobacter pylori* infection: review of the literature. World J Gastroenterol. 2014;20:5205–11.
81. Yahav J, Samra Z, Niv Y, Evans CT, Passaro DJ, Dinari G, et al. Susceptibility-guided vs. empiric retreatment of *Helicobacter pylori* infection after treatment failure. Dig Dis Sci. 2006;51:2316–21.

49

Fluoroquinolone and Rifabutin-Containing Therapy

Jung Won Lee

Abstract

Fluoroquinolone-containing therapy and rifabutin-containing therapy are regimens which use proton pump inhibitor and amoxicillin as it is in clarithromycin-containing triple therapy and replaces clarithromycin to levofloxacin, moxifloxacin, or rifabutin, respectively. These regimens are mainly recommended treatments which we can consider as rescue therapies when first- and second-line eradication therapies fail in *Helicobacter pylori* treatment. Fluoroquinolone-containing triple therapy is known to show favorable eradication rate and lower adverse events rate compared to bismuth quadruple therapy. However, fluoroquinolone-containing therapy is challenged by steep increase in resistance and subsequent decrease in efficacy. In summary, the antimicrobial resistance to rifabutin is still very low. Nevertheless, it has not been proven whether the eradication efficacy of rifabutin-containing triple therapy is comparable to other alternatives, and still there is a possibility can cause serious side effects, such as bone marrow suppression.

Keywords

Helicobacter pylori · Eradication
Fluoroquinolone · Levofloxacin
Moxifloxacin · Rifabutin

49.1 Introduction

When first- and second-line eradication therapies fail in *Helicobacter pylori* (*H. pylori*) treatment, the most frequently recommended regimens as rescue therapy are fluoroquinolone-containing therapy and rifabutin-containing therapy [1, 2]. These therapies are regimens which use proton pump inhibitor (PPI) and amoxicillin as it is in clarithromycin-containing triple therapy and replaces clarithromycin to levofloxacin, moxifloxacin, or rifabutin, respectively. Recent European guideline recommends fluoroquinolone-containing triple or quadruple therapy and rifabutin-containing therapy as second or third-line therapy for *H. pylori* [2]. Guidelines from Asian countries also have similar recommendations on fluoroquinolone-containing therapy and rifabutin-containing therapy as rescue therapy [3]. The circumstances of the USA are different from that of Europe and Asia; the guideline of the USA regards fluoroquinolone-containing therapy and rifabutin-containing triple therapy as rescue therapies, but it does not recommend them positively [4]. The reason might be due to the limitation of

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evidences regarding these regimens in the USA. Like this, although there are some differences in detail, usual guidelines regarded fluoroquinolone- or rifabutin-containing therapies as one of the main rescue therapies. In this chapter, we will review current status regarding efficacy, adverse event, and future perspectives of fluoroquinolone-containing therapy and rifabutin-containing rescue eradication therapy.

49.2 Fluoroquinolone-Containing Triple Therapy

49.2.1 Theoretical Background

Levofloxacin and moxifloxacin are antimicrobial agents of fluoroquinolone family and have antibacterial efficacy by inhibiting DNA gyrase and topoisomerase IV [5]. Levofloxacin shows wide range of antimicrobial effects on gram-positive bacteria, gram-negative bacteria, and atypical respiratory pathogen. Moxifloxacin also demonstrates excellent antimicrobial efficacy against more gram-negative organism. These antimicrobial agents have received attention since their anti-*H. pylori* efficacy was demonstrated in the laboratory [6]. Moreover, after levofloxacin was known to be effective for *H. pylori* strains which have resistance to clarithromycin and metronidazole through laboratory and clinical studies, many studies were performed to explore the efficacy of levofloxacin and moxifloxacin-containing rescue therapy against *H. pylori* [7, 8].

49.2.2 Eradication Rate

Levofloxacin-containing triple therapy usually consists of PPI and amoxicillin and levofloxacin 250–500 mg/day instead of clarithromycin in conventional clarithromycin-containing triple therapy. For moxifloxacin-containing regimen, the dosage is 400 mg/day. The duration of this regimen is 7–14 days. Two systematic reviews regarding levofloxacin-based triple therapy as rescue therapy for *H. pylori* infection were published in 2006. In a systematic review which included four randomized controlled trials for 391 subjects, 10-day levofloxacin-containing triple therapy was superior to 7-day bismuth quadruple therapy (mean eradication rate 87% [95% CI, 82–92%] vs. 68% [95% CI, 62–74%]; relative risks [RR] for successful eradication 1.41; 95% CI, 1.25–1.59) [9]. On the other hand, adverse events (RR 0.51; 95% CI, 0.34–0.75) and drop out by adverse events (RR 0.30; 95% CI, 0.10–0.89) of levofloxacin-containing triple therapy were significantly lower than those of bismuth quadruple therapy. In the subgroup analysis, the eradication rate of levofloxacin-containing triple therapy was higher in 10-day than in 7-day (mean eradication rate 87% [95% CI, 82–92%] vs. 68% [95% CI, 62–74%]). In the aspect of dosage, there was no significant difference in the eradication rate between 500 mg q.d. (quaque die, every day) and 250 mg b.i.d. (bis in die, twice a day) of levofloxacin. The authors concluded that 10-day triple therapy containing levofloxacin 500 mg q.d. was superior to 7-day bismuth quadruple therapy in the aspect of eradication rate and compliance. Another systematic review which included 977 subjects and 14 studies also reported that levofloxacin-containing triple therapy was superior to bismuth quadruple therapy in the eradication rate (81 vs. 70%; odds ratio [OR] 1.80; 95% CI, 0.94–3.46) and levofloxacin-containing triple therapy showed less adverse events (19% vs. 44%; OR 0.27; 95% CI, 0.16–0.46) [10]. In this systematic review, the eradication rate of 10-day levofloxacin-containing triple therapy was higher than that of 7-day levofloxacin-containing triple therapy (81 vs. 73%, $p < 0.01$).

There are also several results from moxifloxacin-containing triple therapy. In a systematic review which included four randomized controlled trials regarding 772 subjects, the eradication rate of moxifloxacin-containing triple therapy was significantly higher than that of clarithromycin-containing triple therapy (mean eradication rate, 84.1% [318/378] vs. 73.6% [290/394]; RR 1.13; 95% CI, 1.01–1.27; $p = 0.04$) [11]. There was no significant difference in the adverse events between the two regimens (RR

0.61; 95% CI, 0.25–1.48; $p < 0.28$). A systematic review which compared moxifloxacin-containing triple therapy and bismuth quadruple therapy for second-line eradication therapy for *H. pylori* infection in 787 subjects and seven randomized controlled trials also reported that moxifloxacin-containing triple therapy is superior to bismuth quadruple therapy in the eradication rate (74.9 vs. 61.4%; OR 1.89; 95% CI, 1.38–2.58; $p < 0.001$) [12]. The rate of adverse events and drop out by adverse events were also lower in moxifloxacin-containing triple therapy than in bismuth quadruple therapy (adverse events rate, 10.1 vs. 27.8%; drug discontinuation rate, 1.4 vs. 8.2%).

49.2.3 Adverse Events

The most common adverse event of levofloxacin is gastrointestinal (GI) symptom such as nausea, diarrhea, and constipation. The second common adverse event is one related to central nervous system such as headache and insomnia. However, most adverse events are mild. Adverse events of moxifloxacin are similar to levofloxacin, and the incidence of adverse events is reported as less than 5% [13]. The reason that the rate of adverse events of triple therapy containing these fluoroquinolones is higher than that of individual fluoroquinolone is that PPI and amoxicillin which are administrated together also cause various adverse events. Therefore, like the aforementioned systematic reviews, it is not arguable that fluoroquinolone-containing triple therapy is superior or at least not inferior to standard triple therapy or bismuth quadruple therapy in the aspect of adverse events and compliance. However, the US-FDA recently added precautions for serious adverse events including rupture of aorta, rupture of tendons, severe hypoglycemia, depression, anxiety, and other mental health issues, which can be caused by fluoroquinolone antimicrobial agents. There is a possibility that restrictions on the use of fluroquinolone will be added. Therefore, use of fluoroquinolones should be cautious unless the therapeutic benefits outweigh the risks.

49.2.4 Antibiotic Resistance and Limitations

Although European guideline recommends levofloxacin-containing triple therapy as one of second-line treatments for *H. pylori* infection, it warned of steeply increasing resistance rate [1, 2]. Actually, resistance to fluoroquinolone is already a severe problem in many countries [14–16]. For example, the eradication rate of first-line and second-line fluoroquinolone-containing therapy in Korea was 69.8% [17] and 51.6–65.5% [17, 18], respectively; these are much lower than those from earlier systematic reviews which evaluated the results from mostly European studies. These disappointing eradication rates of fluoroquinolone-containing therapy in Korea are related to increase in fluoroquinolone resistance during the period. The first and second resistance rate to levofloxacin and moxifloxacin was 4.7% and 16.7%, respectively, during 2003 and 2005; they sharply increased to 28.1% and 50.0% during 2009 and 2012 [19]. And these increasing trends are also confirmed at recent period up to 35.7% in 2022 in Korea [15]. Fluoroquinolone resistance in *H. pylori* strain is usually acquired by mutation of *gyrA* gene [20]. The steep increase of antibiotic resistance and subsequent decrease of eradication rate in Korea are presumed to be related to frequent administration of these antibiotics for respiratory and otolaryngologic diseases [19, 21]. Meanwhile, several investigators focusing on anti-*H. pylori* efficacy of bismuth began to study fluoroquinolone-containing quadruple therapy by adding bismuth to the existing fluoroquinolone-containing triple therapy [22]. Bismuth-containing fluoroquinolone quadruple therapy after failure of first-line non-bismuth quadruple therapy, such as sequential and concomitant therapy, demonstrated more favorable outcomes than conventional bismuth quadruple therapy [16, 22]. In particular, in a systematic review that included 26 recently published studies, fluoroquinolone quadruple therapy showed a high eradication success rate of 90% in 10 days [23–25].

Finafloxacin is a newly developed fluoroquinolone known to be stable in acidic circumstance. In a study published in 2015, anti-*H. pylori* efficacy was demonstrated as a better minimum inhibitory concentration (MIC) value in in vitro acidic conditions compared to moxifloxacin [26]. Sitafloxacin is also a newly developed fluoroquinolone that is currently available in Japan and Thailand [27]. In particular, sitafloxacin is known to maintain eradication efficacy even in the presence of *gyrA* mutations [27, 28]. Several studies have suggested that the *gyrA* mutation is associated with eradication failure [29, 30]. Recently, there is a study that pretreatment determination on *gyrA* mutation using DPO-PCR method can improve eradication efficacy [31]. Molecular determination before fluoroquinolon-containing therapy is also likely to be recommended at near future like clarithromycin-containing therapy. If next-generation sequencing on *H. pylori* isolates becomes common, it is possibly predicted to contribute to improve eradication efficacy of fluoroquinolone-containing therapy as well [32]. In summary, we should be cautious to use fluoroquinolone-containing triple therapy in patients who have history of these diseases, and there are some limitations to use fluoroquinolone-containing triple therapy as second-line treatment for *H. pylori* infection in countries where the resistance rate of fluoroquinolone is over 30%.

49.3 Rifabutin-Containing Therapy

49.3.1 Theoretical Background

Rifabutin is one of rifamycin derivatives. This antibiotics is structurally similar to rifampin (rifampicin) which has most potent antituberculous efficacy and shares many characteristics with rifampin [33]. Mechanism of bactericidal efficacy of rifabutin is inhibiting β-subunit of DNA-dependent RNA polymerase which is encoded by *rpoB* gene in *H. pylori* strain [34]. Rifabutin is used to treat Mycobacterium avium-intracellulare complex (MAC) infections that commonly occur in severely immunosuppressed patients such as AIDS [35, 36]. The reason why rifabutin emerged as a candidate of rescue eradication therapy in *H. pylori* treatment is that this agent shows no cross-resistance with clarithromycin, metronidazole, and fluoroquinolones [36]. In addition, since rifabutin has been used only in AIDS patients and some tuberculosis patients, it is assumed that there is little or no opportunity to induce widespread resistance in *H. pylori* [37]. Moreover, it is also well known that rifabutin still maintains their antimicrobial efficacy even in low pH circumstances in the stomach [38, 39].

49.3.2 Eradication Rate

Rifabutin was initially tried as a triple therapy combined with PPI and amoxicillin [36]. Related studies were mostly performed after 2000 and systematic review was initially published at 2012 [33]. Many studies were performed in European countries, especially in Germany, Italy, and Spain. The dosage is usually 300 mg/day (150 mg b.i.d. or 300 mg q.d.) and treatment duration was mostly 7 or 10 days. The number of subjects in most studies was less than 100, and all studies were regarding over second-line and third-line treatment. When the data regarding 21 studies and 1008 subjects were analyzed, eradication rate by intention-to-treat (ITT) analysis was mean 73% (95% CI, 67–79%). In the subgroup analysis for 223 patients who underwent rifabutin-containing triple therapy as second-line treatment for *H. pylori* infection, eradication rate was mean 79% (95% CI, 67–92%); the eradication rate was higher in studies with prolonged administration (10–12 days) than in the studies with 7-day administration (92 vs. 69%). In the subgroup analysis for 342 patients who underwent rifabutin-containing triple therapy as third-line treatment, eradication rate by ITT analysis was mean 66% (95% CI, 55–77%). There are few studies which directly compared rifabutin-containing triple therapy with other regimens. There are three studies which compared rifabutin-containing triple therapy with bismuth quadruple therapy as second-line treatment for *H. pylori* infection. However, the results were heterogenous [40–42]. There are just two studies which compared rifabutin-containing triple therapy with levofloxacin-containing triple therapy as third-line treatment. First, the Spanish study compared the efficacy of 10-day rifabutin-containing triple therapy and levofloxacin-containing triple therapy in 40 patients who failed second-line treatment of *H. pylori*; the eradication rate by ITT analysis was 45% and 85%, respectively [43]. The authors reported that levofloxacin-containing triple therapy is superior to rifabutin-containing triple therapy. However, this study was open-labeled study and the number of subjects was too small and the eradication rate of rifabutin-containing triple therapy group was unexpectedly low. Another study result was reported in Korea at 2012 [44]. The eradication rate of rifabutin-containing triple therapy and levofloxacin-containing triple therapy was 71.4% and 57.1%, respectively; there was no significant difference in the eradication rate between two regimens ($p = 0.656$). However, the total sample size of this study was just over 20 and the study design was retrospective. In addition, although the treatment duration of levofloxacin-containing triple therapy was constant as 10 days, treatment duration of rifabutin-containing triple therapy varied as 7–14 days.

Follow-up studies on the eradication efficacy of rifabutin were also published as systematic reviews in 2015 and 2020, respectively. Both studies showed ITT eradication rates of 71.8% and 73%, respectively [35, 45]. All included studies were conducted in subjects with a history of previous eradication therapy failure. Thus, rifabutin-containing treatment has been studied only as a rescue therapy. However, a recent study on subjects without previous eradication experience showed an eradication rate of about 80% [46, 47]. Recently, it has been reported that when potassium-competitive acid blocker (PCAB) is used instead of the conventional PPI, effective eradication results (91.2% in ITT and 92.7% in PP) can be achieved even with a low dose of 150 mg/day of rifabutin for the patients with second-line therapy failure [48]. Opinions on optimizing rifabutin-containing therapy have recently been proposed [35]. Optimal results were mostly achieved at 300 mg/day, although some studies reported favorable outcomes even at 150 mg/day. Vonoprazan can also be used. Although 10-day treatment is more effective than 7-day treatment, it has not been concluded whether a 14-day extension is more effective. Also, the results of the European registry suggest that the addition of bismuth may show additional eradication efficacy [49].

49.3.3 Adverse Events

The most common adverse events of rifabutin are rash and GI symptoms such as nausea, vomiting, and dyspepsia. Besides, it can cause neutropenia or anemia and the color of urine can be changed as red [50]. These adverse events are known to be dose-dependent [51]. According to the systematic review, the rate of adverse events in *H. pylori* eradication therapy was mean 22% (95% CI, 19–25%) [33]. Bone marrow suppression is not frequent but often severe adverse event. However, it is known that this adverse event is not common, and it usually occurs when high dose above 600 mg/day is administrated and the treatment duration is long. For *H. pylori* eradication therapy, the rate of bone marrow suppression was reported as 1.5–3.0% in some studies. However, leukopenia was usually recovered spontaneously in a few days, and there was no case in which it causes serious problem. Infection or adverse events related with leukopenia during the use of rifabutin in *H. pylori* eradication therapy have not been reported to date. However, because the total number of tested subjects is still just around one thousand, there is a possibility that these adverse events occur in the future. Therefore, physician should make sufficient explanation for adverse events to the patient before trying rifabutin-containing triple therapy. In addition, they should check complete blood count when symptoms like fever occur and bone marrow suppression are suspected during treatment. Besides, uveitis was reported when rifabutin was administrated together with clarithromycin, ethambutol, or fluconazole [52]. However, this adverse event is common in immunocompromised patients or in patients who received antituberculous treatment for a long period. There has been no case report regarding *H. pylori* eradication treatment until recently [35].

49.3.4 Antibiotic Resistance and Limitations

According to the review articles, primary resistance of *H. pylori* for rifabutin was very low as 0.6–1.4% [2, 14, 53]. Considering that *H. pylori* can acquire resistance to rifabutin by point mutation of *rpoB* gene [54], the reason that resistance rate of *H. pylori* to rifabutin is very low is that these antibiotics have been used clinically for the very limited indications. Therefore, if rifabutin were used widely for *H. pylori* treatment, similar to other antibiotics, there is a possibility that resistance rate increases in the future.

In addition, because rifabutin and rifampicin inhibit *H. pylori* by the same mechanism and they show cross-resistance, if a patient has a history of previous exposure to rifampicin due to the reason such as antituberculous treatment, the possibility that the effect for eradication decreases is high [55]. On the other hand, this is still a considerable limitation in the countries which have high incidence of tuberculosis with concern associated with the use of rifabutin in the development of resistance to *Mycobacterium tuberculosis*. However, no correlation has been reported between the short-term use of rifabutin for non-tuberculosis infections such as *H. pylori* and the development of resistance [56]. In brief, although rifabutin is a promising candidate for rescue eradication, the history of rifampicin containing treatment with any other indications should be carefully considered before rifabutin-containing *H. pylori* eradication [55].

In addition, this drug is expensive, and we should be cautious about the fact that sometimes serious leukopenia or thrombocytopenia was reported. Even though there has been no report regarding resistance induction after short administration such as in *H. pylori* treatment, if rifabutin were generally used for *H. pylori* infection, there is a possibility that resistance of *M. tuberculosis* for rifabutin increases.

49.4 Conclusions

Fluoroquinolone-containing therapy and rifabutin-containing therapy are preferred regimens which we can consider as rescue therapies when first- and second-line eradication therapies failed at *H. pylori* eradication treatment. However, fluroquinolone-containing triple therapy is challenged by steep increase in resistance and subsequent decrease in eradication rate. With regard to rifabutin-containing therapy, although resistance rate is very low at the present, whether eradication rate is superior to other regimens is unclear and there is a possibility of fatal adverse events such as bone marrow suppression.

References

1. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of *Helicobacter pylori* infection--the Maastricht IV/ Florence consensus report. Gut. 2012;61:646–64.
2. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
3. Fock KM, Katelaris P, Sugano K, Ang TL, Hunt R, Talley NJ, et al. Second Asia-Pacific consensus guidelines for *Helicobacter pylori* infection. J Gastroenterol Hepatol. 2009;24:1587–600.
4. Chey WD, Wong BC. Practice Parameters Committee of the American College of G. American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection. Am J Gastroenterol. 2007;102:1808–25.
5. Croom KF, Goa KL. Levofloxacin: a review of its use in the treatment of bacterial infections in the United States. Drugs. 2003;63:2769–802.
6. Sanchez JE, Saenz NG, Rincon MR, Martin IT, Sanchez EG, Martinez MJ. Susceptibility of *Helicobacter pylori* to mupirocin, oxazolidinones, quinupristin/dalfopristin and new quinolones. J Antimicrob Chemother. 2000;46:283–5.
7. Watanabe Y, Aoyama N, Shirasaka D, Maekawa S, Kuroda K, Miki I, et al. Levofloxacin based triple

therapy as a second-line treatment after failure of *Helicobacter pylori* eradication with standard triple therapy. Dig Liver Dis. 2003;35:711–5.

8. Bilardi C, Dulbecco P, Zentilin P, Reglioni S, Iiritano E, Parodi A, et al. A 10-day levofloxacin-based therapy in patients with resistant *Helicobacter pylori* infection: a controlled trial. Clin Gastroenterol Hepatol. 2004;2:997–1002.
9. Saad RJ, Schoenfeld P, Kim HM, Chey WD. Levofloxacin-based triple therapy versus bismuth-based quadruple therapy for persistent *Helicobacter pylori* infection: a meta-analysis. Am J Gastroenterol. 2006;101:488–96.
10. Gisbert JP, Morena F. Systematic review and meta-analysis: levofloxacin-based rescue regimens after *Helicobacter pylori* treatment failure. Aliment Pharmacol Ther. 2006;23:35–44.
11. Wenzhen Y, Kehu Y, Bin M, Yumin L, Quanlin G, Donghai W, et al. Moxifloxacin-based triple therapy versus clarithromycin-based triple therapy for first-line treatment of *Helicobacter pylori* infection: a meta-analysis of randomized controlled trials. Intern Med. 2009;48:2069–76.
12. Wu C, Chen X, Liu J, Li MY, Zhang ZQ, Wang ZQ. Moxifloxacin-containing triple therapy versus bismuth-containing quadruple therapy for second-line treatment of *Helicobacter pylori* infection: a meta-analysis. Helicobacter. 2011;16:131–8.
13. Mandell L, Tillotson G. Safety of fluoroquinolones: an update. Can J Infect Dis. 2002;13:54–61.
14. Megraud F, Bruyndonckx R, Coenen S, Wittkop L, Huang TD, Hoebeke M, et al. *Helicobacter pylori* resistance to antibiotics in Europe in 2018 and its relationship to antibiotic consumption in the community. Gut. 2021;70:1815–22.
15. Lee JW, Kim N, Choi SI, Jang JY, Song CH, Nam RH, et al. Prevalence and trends of multiple antimicrobial resistance of *Helicobacter pylori* in one tertiary hospital for 20 years in Korea. Helicobacter. 2023;28:e12939.
16. Gisbert JP. Optimization strategies aimed to increase the efficacy of *Helicobacter pylori* eradication therapies with quinolones. Molecules. 2020;25:5084.
17. Lee JH, Hong SP, Kwon CI, Phyun LH, Lee BS, Song HU, et al. The efficacy of levofloxacin based triple therapy for *Helicobacter pylori* eradication. Korean J Gastroenterol. 2006;48:19–24.
18. Kang MS, Park DI, Yun JW, Oh SY, Yoo TW, Park JH, et al. Levofloxacin-azithromycin combined triple therapy for *Helicobacter pylori* eradication. Korean J Gastroenterol. 2006;47:30–6.
19. Lee JW, Kim N, Kim JM, Nam RH, Chang H, Kim JY, et al. Prevalence of primary and secondary antimicrobial resistance of *Helicobacter pylori* in Korea from 2003 through 2012. Helicobacter. 2013;18:206–14.
20. Moore RA, Beckthold B, Wong S, Kureishi A, Bryan LE. Nucleotide sequence of the *gyrA* gene and characterization of ciprofloxacin-resistant mutants of *Helicobacter pylori*. Antimicrob Agents Chemother. 1995;39:107–11.
21. Liao J, Zheng Q, Liang X, Zhang W, Sun Q, Liu W, et al. Effect of fluoroquinolone resistance on 14-day levofloxacin triple and triple plus bismuth quadruple therapy. Helicobacter. 2013;18:373–7.
22. Chang YL, Tung YC, Tu YK, Yeh HZ, Yang JC, Hsu PI, et al. Efficacy of second-line regimens for *Helicobacter pylori* eradication treatment: a systemic review and network meta-analysis. BMJ Open Gastroenterol. 2020;7:e000472.
23. Gisbert JP, Romano M, Gravina AG, Solis-Munoz P, Bermejo F, Molina-Infante J, et al. *Helicobacter pylori* second-line rescue therapy with levofloxacin- and bismuth-containing quadruple therapy, after failure of standard triple or non-bismuth quadruple treatments. Aliment Pharmacol Ther. 2015;41:768–75.
24. Yeo YH, Hsu CC, Lee CC, Ho HJ, Lin JT, Wu MS, et al. Systematic review and network meta-analysis: comparative effectiveness of therapies for second-line *Helicobacter pylori* eradication. J Gastroenterol Hepatol. 2019;34:59–67.
25. Marin AC, Nyssen OP, McNicholl AG, Gisbert JP. Efficacy and safety of quinolone-containing rescue therapies after the failure of non-bismuth quadruple treatments for *Helicobacter pylori* eradication: systematic review and meta-analysis. Drugs. 2017;77:765–76.
26. Lee JW, Kim N, Nam RH, Kim JM, Park JY, Lee SM, et al. High efficacy of finafloxacin on *Helicobacter pylori* isolates at pH 5.0 compared with that of other fluoroquinolones. Antimicrob Agents Chemother. 2015;59:7629–36.
27. Mori H, Suzuki H. Update on quinolone-containing rescue therapies for *Helicobacter pylori* infection. World J Gastroenterol. 2020;26:1733–44.
28. Mori H, Suzuki H, Matsuzaki J, Tsugawa H, Fukuhara S, Miyoshi S, et al. Efficacy of 10-day sitafloxacin-containing third-line rescue therapies for *Helicobacter pylori* strains containing the *gyrA* mutation. Helicobacter. 2016;21:286–94.
29. Lee JW, Kim N, Nam RH, Park JH, Kim JM, Jung HC, et al. Mutations of *Helicobacter pylori* associated with fluoroquinolone resistance in Korea. Helicobacter. 2011;16:301–10.
30. Lee JW, Kim N, Nam RH, Park JH, Choi YJ, Kim JM, et al. GenoType HelicoDR test in the determination of antimicrobial resistance of *Helicobacter pylori* in Korea. Scand J Gastroenterol. 2014;49:1058–67.
31. Haumaier F, Schneider-Fuchs A, Backert S, Vieth M, Sterlacci W, Wohrl BM. Rapid detection of quinolone resistance mutations in *gyrA* of *Helicobacter pylori* by real-time PCR. Pathogens. 2022;11:59.
32. Cummings LC, Hojat LS, Nguyen DC, Stempak LM, Rhoads DD, Sadri N. Tailored treatment based on *Helicobacter pylori* genetic markers of resistance is associated with higher eradication success. Am J Gastroenterol. 2023;118:360–3.
33. Gisbert JP, Calvet X. Review article: rifabutin in the treatment of refractory *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2012;35:209–21.

34. Heep M, Beck D, Bayerdorffer E, Lehn N. Rifampin and rifabutin resistance mechanism in *Helicobacter pylori*. Antimicrob Agents Chemother. 1999;43:1497–9.
35. Gisbert JP. Rifabutin for the treatment of *Helicobacter pylori* infection: a review. Pathogens. 2020;10:15.
36. Perri F, Festa V, Clemente R, Quitadamo M, Andriulli A. Rifabutin-based 'rescue therapy' for *Helicobacter pylori* infected patients after failure of standard regimens. Aliment Pharmacol Ther. 2000;14:311–6.
37. Megraud F, Lamouliatte H. Review article: the treatment of refractory *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2003;17:1333–43.
38. Akada JK, Shirai M, Fujii K, Okita K, Nakazawa T. In vitro anti-*Helicobacter pylori* activities of new rifamycin derivatives, KRM-1648 and KRM-1657. Antimicrob Agents Chemother. 1999;43:1072–6.
39. Koudriakova T, Iatsimirskaia E, Tulebaev S, Spetie D, Utkin I, Mullet D, et al. In vivo disposition and metabolism by liver and enterocyte microsomes of the antitubercular drug rifabutin in rats. J Pharmacol Exp Ther. 1996;279:1300–9.
40. Perri F, Festa V, Clemente R, Villani MR, Quitadamo M, Caruso N, et al. Randomized study of two "rescue" therapies for *Helicobacter pylori*-infected patients after failure of standard triple therapies. Am J Gastroenterol. 2001;96:58–62.
41. Wong WM, Gu Q, Lam SK, Fung FM, Lai KC, Hu WH, et al. Randomized controlled study of rabeprazole, levofloxacin and rifabutin triple therapy vs. quadruple therapy as second-line treatment for *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2003;17:553–60.
42. Navarro-Jarabo JM, Fernandez N, Sousa FL, Cabrera E, Castro M, Ramirez LM, et al. Efficacy of rifabutin-based triple therapy as second-line treatment to eradicate *Helicobacter pylori* infection. BMC Gastroenterol. 2007;7:31.
43. Gisbert JP, Gisbert JL, Marcos S, Moreno-Otero R, Pajares JM. Third-line rescue therapy with levofloxacin is more effective than rifabutin rescue regimen after two *Helicobacter pylori* treatment failures. Aliment Pharmacol Ther. 2006;24:1469–74.
44. Jeong MH, Chung JW, Lee SJ, Ha M, Jeong SH, Na S, et al. Comparison of rifabutin- and levofloxacin-based third-line rescue therapies for *Helicobacter pylori*. Korean J Gastroenterol. 2012;59:401–6.
45. Liu X, Wang H, Lv Z, Wang Y, Wang B, Xie Y, et al. Rescue therapy with a proton pump inhibitor plus amoxicillin and rifabutin for *Helicobacter pylori* infection: a systematic review and meta-analysis. Gastroenterol Res Pract. 2015;2015:415648.
46. Graham DY, Canaan Y, Maher J, Wiener G, Hulten KG, Kalfus IN. Rifabutin-based triple therapy (RHB-105) for *Helicobacter pylori* eradication: a double-blind, randomized, controlled trial. Ann Intern Med. 2020;172:795–802.
47. Gingold-Belfer R, Niv Y, Levi Z, Boltin D. Rifabutin triple therapy for first-line and rescue treatment of *Helicobacter pylori* infection: a systematic review and meta-analysis. J Gastroenterol Hepatol. 2021;36:1392–402.
48. Inokuchi K, Mori H, Matsuzaki J, Hirata K, Harada Y, Saito Y, et al. Efficacy and safety of low-dose rifabutin-based 7-day triple therapy as a third- or later-line *Helicobacter pylori* eradication regimen. Helicobacter. 2022;27:e12900.
49. Nyssen OP, Vaira D, Saracino IM, Fiorini G, Caldas M, Bujanda L, et al. Experience with rifabutin-containing therapy in 500 patients from the European Registry on *Helicobacter pylori* Management (Hp-EuReg). J Clin Med. 2022;11:1658.
50. Brughera M, Scampini G, Newman AJ, Castellino S, Sammartini U, Mazue G. Overview of toxicological data on rifabutin. Exp Toxicol Pathol. 1995;47:1–9.
51. Brogden RN, Fitton A. Rifabutin. A review of its antimicrobial activity, pharmacokinetic properties and therapeutic efficacy. Drugs. 1994;47:983–1009.
52. Skolik S, Willermain F, Caspers LE. Rifabutin-associated panuveitis with retinal vasculitis in pulmonary tuberculosis. Ocul Immunol Inflamm. 2005;13:483–5.
53. Ho JJC, Navarro M, Sawyer K, Elfanagely Y, Moss SF. *Helicobacter pylori* antibiotic resistance in the United States between 2011 and 2021: a systematic review and meta-analysis. Am J Gastroenterol. 2022;117:1221–30.
54. Heep M, Rieger U, Beck D, Lehn N. Mutations in the beginning of the *rpoB* gene can induce resistance to rifamycins in both *Helicobacter pylori* and *mycobacterium tuberculosis*. Antimicrob Agents Chemother. 2000;44:1075–7.
55. Suzuki S, Suzuki H, Nishizawa T, Kaneko F, Ootani S, Muraoka H, et al. Past rifampicin dosing determines rifabutin resistance of *Helicobacter pylori*. Digestion. 2009;79:1–4.
56. Van der Poorten D, Katelaris PH. The effectiveness of rifabutin triple therapy for patients with difficult-to-eradicate *Helicobacter pylori* in clinical practice. Aliment Pharmacol Ther. 2007;26:1537–42.

Probiotics

50

Nayoung Kim and Sung Wook Hwang

Abstract

Probiotics have been used for decades to alleviate the side effects of oral antibiotics. Various microorganisms are used as probiotics, of which lactic acid-producing bacteria such as *Lactobacillus* spp., *Bifidobacterium* spp., etc. are the most commonly used. The effects of probiotics are carried out through immunological or nonimmunological mechanisms. Production of antibiotic substances, competition with *Helicobacter pylori* (*H. pylori*) for adhesion receptors, stimulation of mucin production, stabilization of the gut mucosal barrier, etc. are being suggested as nonimmunological mechanisms. It is true that improvement in *H. pylori* eradication rate, decrease in antibiotic-associated side effects, reduced *H. pylori* colonization, and decreased *Helicobacter*-induced gastritis are being reported in human studies with probiotics. Recently, there have been numerous reports regarding the effects of gut microbiome in our body, thus probiotics became very important for the physicians. However, mechanistic understanding on how they work is so far incomplete. Thus, further investigation is needed as results are shown to differ among studies.

Keywords

Helicobacter pylori · Probiotics
Lactobacillus · *Bifidobacterium* · Eradication
Side effects

50.1 Introduction

Probiotics are “live, nonpathogenic microbial feeds or food supplements that exert a positive influence on their host by altering his microbial balance” [1]. Known as the “father of lactic acid bacteria,” Nobel Prize-winning Metchnikoff hypothesized in *The Prolongation of Life* in 1907 that indigested food and feces in the intestine produce toxin and shorten one’s life and mentioned that lactic acid-fermented milk diminishes toxin and supports prolongation of human life based on the fact that there are many people who live long in Bulgaria and the Caucasus where people regularly consume lactic acid-fermented milk. Currently, various microorganisms are used as probiotics, and the most commonly used probiotic is lactic acid-producing *Lactobacillus* spp.. Others include *Bifidobacterium* spp., *Bacillus*

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spp., *Escherichia coli*, *Saccharomyces boulardii*, *Streptococcus thermophilus*, etc. [2]. The biological mechanisms of action of probiotics are known to include synthesis of antimicrobial substances, competitive inhibition of pathogenic bacteria, modification of toxins, immune regulation, etc. [1, 3].

Probiotics have been continuously studied for its treatment for and preventive effects on inflammatory diseases, such as atopy, acute infectious diarrhea, and inflammatory bowel disease. Probiotics are also being reported to be helpful for the treatment against *Helicobacter pylori* (*H. pylori*) infection [1, 4]. In addition, as eradication of *H. pylori* was associated with widespread changes in gut microbial ecology and structure, probiotic supplementation could relieve more gastrointestinal (GI) symptoms by inducing alterations in gut microbiota and host immune responses [5]. The issue of antibiotic resistance is being raised and the expectation on probiotic supplementation has been increased. In this chapter, we have aimed to explore the effect of probiotics against *H. pylori* infection.

50.2 Mechanism of Action of Probiotics on *H. pylori* Infection

Studies on the mechanism of action of probiotics are being conducted through various in vitro studies. Largely, they are classified as immunological mechanism and nonimmunological mechanism. Different probiotics show various immune responses according to the host's immune status [6].

50.2.1 Immunological Mechanisms

Probiotic immunomodulation is achieved through a balance of pro- and anti-inflammatory cytokines, and animal studies have shown that probiotic reduces gastric inflammation [1, 4]. Bacteria binds to receptors on the surface of epithelial cells, such as Toll-like receptors, initiating various immunological mechanisms [7]. Initially, the secretion of pro-inflammatory cytokines, such as interleukin (IL)-8, causes migration of neutrophils and monocytes to the gastric mucosa. Activated monocytes and dendritic cells then secrete a variety of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and IL-6 [8]. Meanwhile, probiotics eventually regulate the immune response and reduce gastric inflammation by interacting with epithelial cells producing anti-inflammatory cytokines [9]. In 1997, Kabir et al. have reported that *L. salivarius* inhibits IL-8 secretion stimulated by *H. pylori* [10]. Several animal studies have shown that probiotics decreased specific IgG antibodies to *H. pylori* [11, 12] and increased IgA production in the intestinal epithelial cells [8, 13]. In addition, it has been reported that large amounts of *L. acidophilus* inactivate Jak1/Stat1 pathway reducing *H. pylori*-induced Smad7 transcription and inhibit NF-κB production leading to a reduction of gastric inflammation [14]. However, it is difficult to generalize these effects on immune responses as probiotics show various responses depending on the host's immune status.

50.2.2 Nonimmunological Mechanisms

Upon entrance of bacteria into the stomach, nonimmunological barrier such as gastric acid and gastric mucosal barrier acts as a first line of defense [15]. Studies have reported probiotics' effect of strengthening these nonimmunological barriers and suggested the production of antimicrobial substances, coaggregation with the bacteria, competing with *H. pylori* for adhesion receptors, stimulating mucin production, and strengthening the gastric mucosal barrier through this mechanism [4].

Several *Lactobacilli* synthesize bacteriocin-related antimicrobial substances, and other substances secreted include those which inhibit bacteria such as organic acids, hydrogen peroxide, and carbon dioxide [16–18]. It has also been reported that large amounts of lactate, which is produced by probiotics, inhibit *H. pylori*. Produced lactate not only shows antimicrobial effects by lowering the pH, but also inhibits urease of *H. pylori* [11]. However, these inhibitory effects differ depending on the strain.

H. pylori adhering to the epithelial cells is the most important phase of developing *H. pylori*-associated disease. In a cell line study, it has been reported that *L. johnsonii* La1, *L. salivarius*, *L. acidophilus*, etc. inhibit *H. pylori* adhesion to the epithelial cell lines [9, 19]. This inhibitory effect is exerted through nonspecific blockage of receptor sites or specific binding to carbohydrate receptors by probiotics [4] or showing antiadhesive activity through antimicrobial substances produced [15].

Mucous produced by epithelial cells combines with bacteria and interferes with the adhesion of bacteria on the epithelial cells. *H. pylori* gastritis often shows reduced mucous secretion from damaged epithelium [20]. It has been reported that *L. plantarum* and *L. rhamnosus* increase the expression of MUC2 and MUC3 and mucous secretion from colon epithelial cells [21, 22].

50.3 Probiotic Treatment of *H. pylori* Infection

L. johnsonii La1 and *L. rhamnosus* GG are most commonly used in probiotic studies in animals and human in addition to *L. casei*, *L. acidophilus*, *L. brevis*, and *Bifidobacterium* [4]. The effects of probiotics on *H. pylori* infection have been assessed mainly through rapid urease test, urea breath test, serology test, stool antigen test, biopsy, etc.

50.3.1 Studies Using Animals and Cell Line

Effects of various probiotics on *H. pylori* infection in animal studies have been reported [10, 12, 23–27]. Oral administration of *L. salivarius* in *H. pylori*-infected BALB/c mice has shown preventive and treatment effects [10, 12]. Also, Johnson-Henry et al. showed reduction in *H. pylori* colonization and *H. pylori*-induced gastritis in a C57BL/6 murine model administered with *L. acidophilus* R0052 and *L. rhamnosus* R0011 [23]. A Mongolian gerbil model also showed the effects of probiotics on *H. pylori* infection through probiotics-induced reduction of *H. pylori* colonization, reduction of mucin infection, and change in blood flow and gastrin-somatostatin [24]. Sgouras et al. reported that administration of *L. casei* strain Shirota and *L. johnsonii* La1 in a C57BL/6 model reduced *H. pylori* gastritis. *L. casei* strain Shirota also reduced *H. pylori* colonization [25]. It has also been shown that in clarithromycin-resistant *H. pylori* infection, *L. gasseri* reduced *H. pylori* colonization [26].

Recently, it has been reported that coculture with *H. pylori* and *Lactobacilli* inhibits *H. pylori* growth and that supernatant lacking cells and living *Lactobacilli* both have anti-*H. pylori* activity [27]. Hwang et al. reported that supernatant produced by the cultivation of *L. acidophilus* and *L. plantarum* reduces *H. pylori* infection in the gastric epithelial cell line [28]. The precise mechanisms of the probiotic bacteria-producing molecules effective for the treatment of *H. pylori* infection are needed to be investigated in the further study.

50.3.2 Human Studies

Results of numerous clinical studies have been presented on the role of probiotics regarding treatment of *H. pylori* infection, and well-systematized studies on relatively large number of adult patients have been summarized [29–53] (Tables 50.1 and 50.2). Probiotics have been studied as supplements to *H. pylori* antibiotic treatment or as substitution for antibiotics, and studies have been mainly focused on its ability to improve the eradication rate. The composition of microbial community across saliva, stomach, and gut changed significantly after eradication, with the gut microbiota swiftly restored to baseline as soon as 2 weeks after cessation of antibiotics [43]. Meanwhile, alterations of oral and gastric microbiota were also observed. Probiotics mitigated the fluctuations of gastric microbiota as well as promoted the enrichment of beneficial bacteria in the mouth [43] (Fig. 50.1).

Table 50.1 Summary of study results using probiotics as *H. pylori* infection treatment.

Probiotics	Publication year	Number of patients	Study design	Results	References
L. acidophilus	1998	15	O	Eradication rate ↑, *H. pylori* colonization ↓	Mrda et al. [42]
L. johnsonii La1	2001	53	DB, P, R	Eradication rate ↔, urease activity ↓, *H. pylori* colonization ↓	Felley et al. [40]
L. johnsonii Lj1	2003	50	DB, P, R	Eradication rate ↔, *H. pylori* colonization ↓, gastritis ↓	Pantoflickova et al. [39]
Lactobacillus- and *Bifidobacterium*-containing yogurt (AB-yogurt)	2004	70	O	Eradication rate ↔, urease activity ↓, *H. pylori* colonization ↓, gastritis ↓	Wang et al. [38]
B. bifidum	2007	79	DB, P, R	Eradication rate ↑, ΔUBT ↓, PG I ↓	Miki et al. [37]
L. reuteri ATCC 55730	2008	40	DB, P, R	Eradication rate ↔, ΔUBT ↓	Francavilla et al. [36]

Modified from Patel et al. [4]
O open label, R randomized, DB double blind, P placebo controlled, ↑ increase, ↓ decrease, ↔ no effect

Recently, another paper proved this transient perturbation of fecal microbiota α-diversity by 16S RNA metagenomics sequencing as well as shotgun sequencing. The perturbation which occurred 2 weeks after two eradication regimens were largely restored to pretreatment state from 8 weeks after eradication. However, diversity recovery was slower with bismuth-based quadruple therapy (BQ10, esomeprazole, bismuth, tetracycline, and metronidazole) for 10 days than levofloxacin-based quadruple therapy (EAML14, esomeprazole, amoxicillin, metronidazole, and levofloxacin) for 14 days [54] (Fig. 50.2).

Interestingly, the disturbance of microbiota induced by eradication drugs could be partially alleviated by probiotics treatment [43]. In addition, probiotic supplementation to antibiotic regimens against *H. pylori* infection has been proposed to improve eradication rate in addition to decrease detrimental effects on gut microbiota [55]. Another matter of interest of studies has been how well probiotics could reduce side effects of antibiotics, such as GI disorders induced by the change in intestinal bacterial flora.

O open label, *R* randomized, *DB* double blind, *P* placebo controlled, *UBT* urea breath test, *PG* pepsinogen, ↑ increase, ↓ decrease, ↔ no effect.

In 2000, Canducci et al. performed the first human study showing improvement of *H. pylori* eradication rate in the patient group treated with *L. acidophilus* LB [35] (Table 50.1). However, it did not alleviate side effects of antibiotics. Afterward, Armuzzi et al. showed reduction of side effects, such as diarrhea and nausea, through *L. rhamnosus* GG administration [34, 56]. Sheu et al. reported that administration of *L. acidophilus* La5 and *Bifidobacterium lactis* Bb12 improves the eradication rate and reduces side effects of antibiotic treatment [41]. Also in Korea, Kim et al. showed improvement in eradication rate with yogurt including *L. acidophilus*, *L. casei*, *Bifidobacterium longum,* and *Streptococcus thermophilus* in addition to the standard triple therapy [31]. Effects of probiotics have also been reported in patients with secondary treatment due to *H. pylori* antibiotics tolerance [29, 30], and Ojetti et al. reported that *L. reuteri* in addition to levofloxacin, amoxicillin, and esomeprazole secondary triple therapy improved the eradication rate and reduced side effects [29].

A few studies have shown the effects of probiotics on *H. pylori* infection in pediatrics [57, 58]. In 2005, Sykora et al. performed a double-blinded randomized clinical study on 86 *H. pylori*-positive pediatrics and showed improvement in eradication rate when given milk with *L. casei* DN-114001 (OAC-LC) in addition to the existing standard triple therapy (clarithromycin, amoxicillin, omeprazole) [58]. In another double-blinded randomized study on 40 pediatric patients, addition

Table 50.2 Summary of study results on probiotics-supplemented *H. pylori* infection treatment

Probiotics	Publication year	*H. pylori* eradication treatment	Number of patients	Study design	Eradication rates	Side effects	References
L. acidophilus LB	2000	Clarithromycin + amoxicillin + PPI	120	O, R	↑	↔	Candducci et al. [35]
Lactobacillus GG	2001	Clarithromycin + tinidazole + PPI	120	DB, P, R	↔	↓	Armuzzi et al. [34]
Lactobacillus- and *Bifidobacterium*-containing yogurt (AB-yogurt)	2002	Clarithromycin + amoxicillin + PPI	160	O, R	↑	↓	Sheu et al. [41]
Lactobacillus GG *Saccharomyces boulardii* *Lactobacillus* spp., *Bifidobacteria*	2002	Clarithromycin + tinidazole + PPI	85	DB, P, R	↔	↓	Cremonini et al. [33]
L. casei subsp. *casei* DG	2004	Bismuth + amoxicillin + tinidazole + PPI (second-line quadruple)	70	R	↔	↓	Tursi et al. [32]
L. acidophilus HY2177, *L. casei* HY2743, *B. longum* HY8001, *Streptococcus thermophilus* B-1 (will yogurt)	2008	Clarithromycin + amoxicillin + PPI	347	O, R	↑	↔	Kim et al. [31]
L. acidophilus HY2177, *L. casei* HY2743, *B. longum* HY8001, *S. thermophilus* B-1 (will yogurt)	2011	Moxifloxacin + amoxicillin + PPI (second-line quadruple)	337	O, R	↔	↔	Yoon et al. [30]
Lactobacillus reuteri	2012	Levofloxacin + amoxicillin + PPI (second-line quadruple)	90	R	↑	↓	Ojetti et al. [29]
B. Tetragenous viable bacteria tablets	2022	Bismuth + amoxicillin + furazolidone + PPI	276	R, DB, P	↔	↓	He et al. [43]
Clostridium butyricum	2018	Bismuth + amoxicillin + furazolidone + PPI	105	O, R	↔	↔	Chen et al. [5]
S. boulardii	2022	Bistmuth + amoxicillin + furazolidone + PPI	100	R	↔	↔	Qu et al. [44]
L. acidophilus LA-5, *Lactiplantibacillus plantarum*, *B. lactis* Bb12, *S. boulardii*	2022	Clarithromycin + metronidazole + amoxicillin + PPI	741	R, DB, P	↑	↓	Viazis et al. [45]

(continued)

Table 50.2 (continued)

Probiotics	Publication year	*H. pylori* eradication treatment	Number of patients	Study design	Eradication rates	Side effects	References
L. casei subsp. casei DG	2015	Levofloxacin + doxycycline + PPI	142	O, R	↔	↔	Paoluzi et al. [46]
Nonviable *L. reuteri* DSM17648	2021	Clarithromycin + amoxicillin + PPI	200	R, DB, P	↔	↓	Yang et al. [47]
L. plantarum CETC7879, *Pediococcus acidilactici*	2018	Clarithromycin + amoxicillin + PPI or Clarithromycin + metronidazole + amoxicillin + PPI	209	R, DB, P	↔	↔	McNicholl et al. [48]
S. boulardii	2022	Bismuth + amoxicillin + calrithromycin + PPI	180	R	↔	↓	He et al. [49]
L. reuteri DSM 17938, *L. reuteri* ATCC PTA 6475 (Gastrus)	2014	Clarithromycin + amoxicillin + PPI	100	R, DB, P	↔	↓	Francavilla et al. [50]
S. boulardii	2020	Clarithromycin + amoxicillin + PPI	217	R	↔	↔	Chang et al. [51]
S. boulardii	2019	Amoxicillin + PPI → clarithromycin + metronidazole + PPI (sequential therapy)	199	O, R	↑	↓	Seddik et al. [52]
Enterococcus faecium, Bacillus subtilis (medlac-S®)	2023	Clarithromycin + amoxicillin + PPI	270	R, DB, P	↔	↓	Lim et al. [53]

PPI proton pump inhibitor, *O* open label, *R* randomized, *DB* double blind, *P* placebo controlled, ↑ increase, ↓ decrease, ↔ no effect

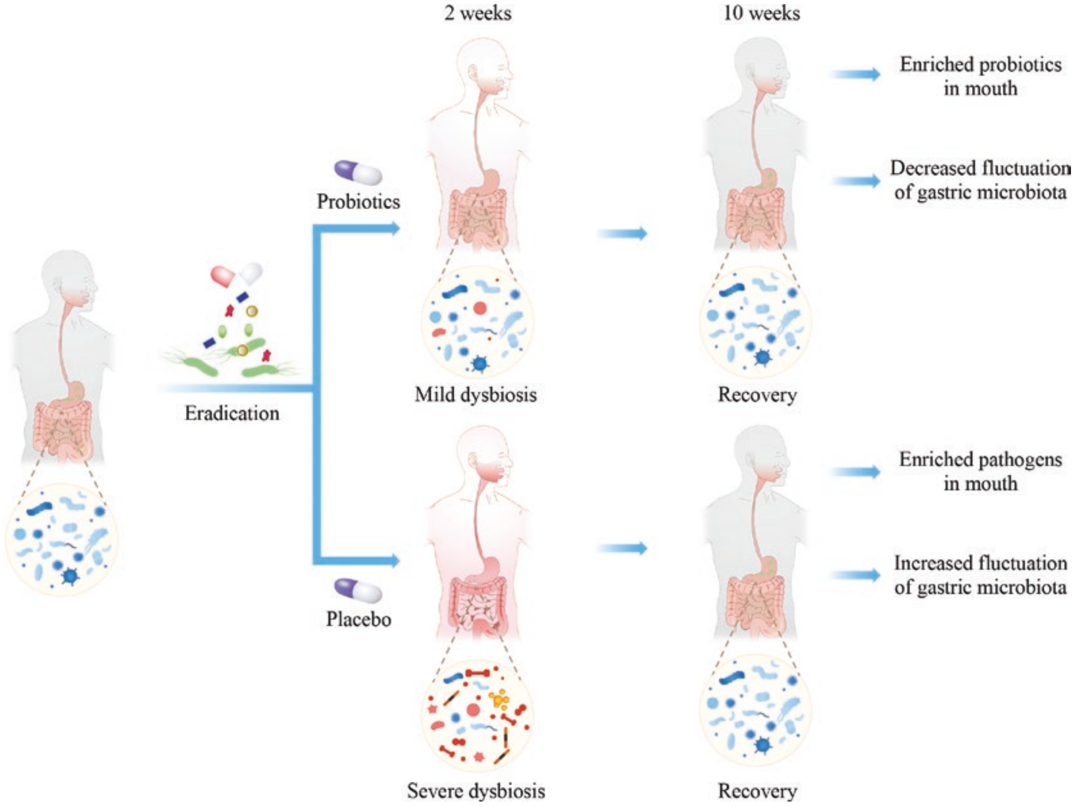
2 weeks
10 weeks
Enriched probiotics in mouth
Decreased fluctuation of gastric microbiota
Probiotics
Eradication
Mild dysbiosis
Recovery
Enriched pathogens in mouth
Increased fluctuation of gastric microbiota
Placebo
Severe dysbiosis
Recovery

Fig. 50.1 Dramatical changes in gut microbiota immediately after *H. pylori* eradication, which were swiftly restored after 8 weeks. These perturbations were partially counteracted by probiotics. (Adapted from He et al. [43])

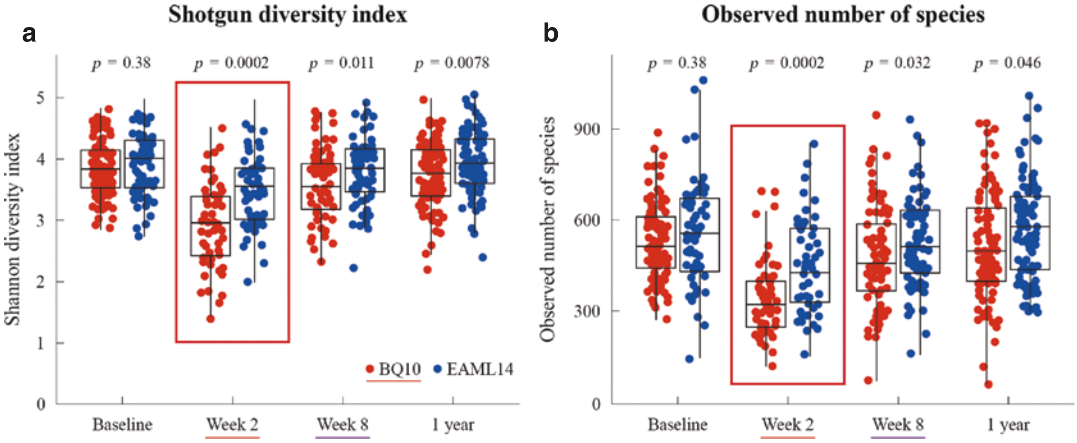

Fig. 50.2 Shotgun a-diversity (**a**) and observed number of gut microbiota after EAML14 and BQ10 (**b**). The perturbation 2 weeks after two eradication regimens were largely restored to pretreatment state from 8 weeks after eradication. However, diversity recovery was slower with bismuth-based quadruple therapy (BQ10, esomeprazole, bismuth, tetracycline, and metronidazole) for 10 days than levofloxacin-based quadruple therapy (EAML14, esomeprazole, amoxicillin, metronidazole, and levofloxacin) for 14 days. (Adapted from Liou et al. [54])

of *L. reuteri* ATCC 55730 to clarithromycin, tinidazole, and omeprazole treatment showed reduced antibiotic-related side effects [57].

Various studies have been conducted on whether probiotics can substitute antibiotic treatment (Table 50.2). In 1998, Mrda et al. performed a study on 15 *H. pylori*-positive patients who were given *L. acidophilus* fermented milk. Among 14 patients who completed the study, 6 patients showed *H. pylori* eradication [42]. Afterward, Michetti et al. reported reduced activity through administration of *L. acidophilus* (*johnsonii*) La1 culture supernatant [59], and two other studies on *L. acidophilus* (*johnsonii*) also showed similar results [39, 40]. However, these studies did not show *H. pylori* eradication effects of probiotics. In a double-blinded randomized study performed by Imase et al., inhibition of *H. pylori* activity and density were shown through urea breath test following administration of *L. reuteri* [60], and Francavilla et al. also reported similar study results on *L. reuteri* [36]. Recently, there was a nice study which shows the side effect of eradication regimen (vonoprazan 20 mg twice daily, amoxicillin 750 mg twice daily, and clarithromycin, 400 mg twice daily) for 7 days and the effect of Biofermin-R (BFR), a multiple antibiotic-resistant lactic acid bacteria preparation of *Enterococcus faecium* 129 BIO 3B-R in normalizing the gut microbiota when used in combination with antibiotics [61]. The relative abundance of gut microbiota larger than 5%. (a) Biofermin-R (BFR) − group and (b) BFR+ group at three time points: before therapy, just after eradication treatment, and after the jugement of eradication [61] (Fig. 50.3a, b). Supplementation with BFR prevented the decrease in α-diversity after eradication therapy (Day 7) [61] (Fig. 50.4a, b).

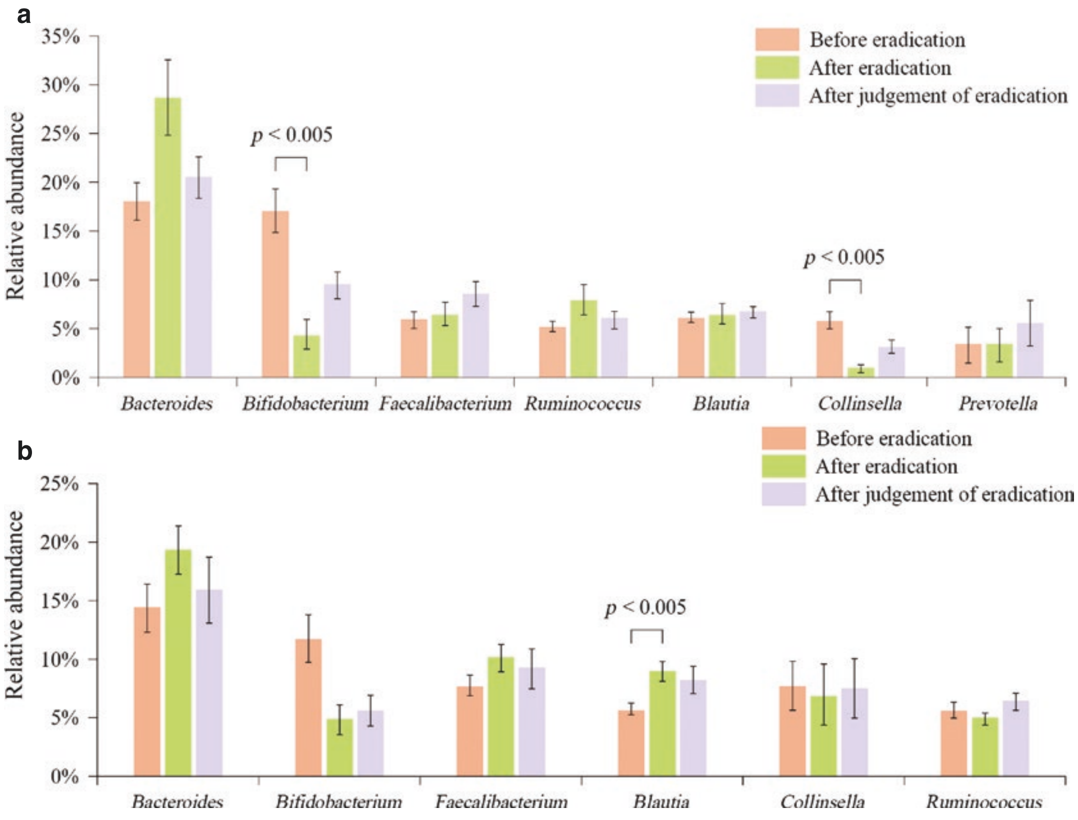

Fig. 50.3 The relative abundance of gut microbiota larger than 5%. (**a**) Biofermin-R (BFR) − group and (**b**) BFR+ group at three time points: before therapy, after eradication, and after the judgment of eradication. There was a difference of relative abundance of stool by vonoprazan containing triple therapy for 7 days and this disappeared by co-treatment of probiotics. (Adapted from Kakiuchi et al. [61])

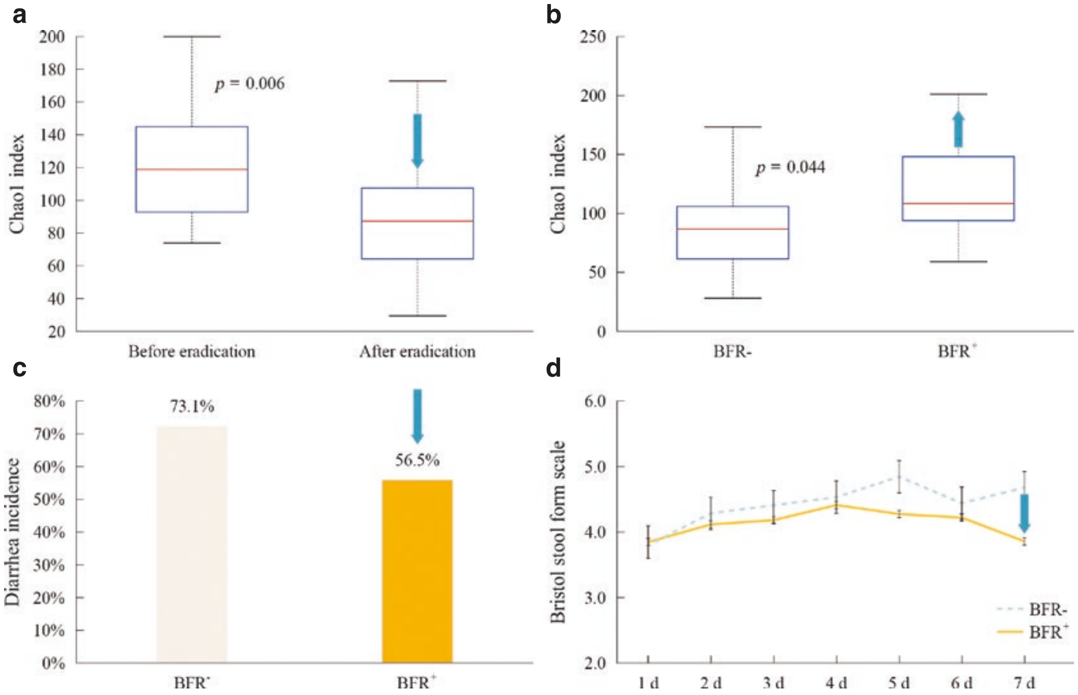

Fig. 50.4 Boxplot depicting Chao1 α-diversity before vonoprazan containing triple eradication therapy and after eradication in the Biofermin-R (BFR) − group (**a**) and after eradication in the BFR− and BFR+ groups (**b**). In addition, the diarrhea and stool analysis. (**c**) Bar diagram showing the incidence rate of diarrhea in the BFR− and BFR+ groups (BFR− group: 73.1% vs. BFR+ group: 56.5%; $p = 0.361$). (**d**) Line diagram represents stool consistency in the Bristol stool form scale. The stool consistency revealed no significant differences between Days 1 and 7 in the BFR+ group; however, a significant difference was observed in the BFR− group between Days 1 and 7 ($p = 0.009$). (Adapted from Kakiuchi et al. [61])

β-diversity was similar between groups. The incidence rate of diarrhea was nonsignificantly higher in the BFR− than in the BFR+ group (73.1% vs. 56.5%; $p = 0.361$) [61] (Fig. 50.4c). Stool consistency was comparable in the BFR+ group on Days 7 and 1 (3.86 ± 0.95 vs. 3.86 ± 1.46; $p = 0.415$) [61] (Fig. 50.4d). These results suggest that Biofermin-R combined with vonoprazan-based therapy resulted in higher microbial α-strain diversity and suppressed stool softening during *H. pylori* eradication therapy [61].

50.4 Safety

Probiotics are known to be safe and regarded as GRAS (generally regarded as safe) by the WHO [4]. In fact, *Lactobacilli* and *Bifidobacteria* are part of normal bacterial flora, and it has been shown that *Bifidobacteria* do not have particular side effects when taken by healthy people and do not have any effects on the immune status [62]. However, in 2008, Besselink et al. reported that probiotics intake by acute pancreatitis patients does not reduce infection complications and rather increase the mortality rate [63] and several *Lactobacilli* bacteremia have also been reported [64]. For this reason, caution is needed when patients take probiotics.

50.5 Conclusions

Studies are conducted on the role of probiotics in the treatment for *H. pylori* infection. As probiotics are produced as medicine and dairy products, they are safe and easy to take and show various effects in human studies. Recently, there were many publications regarding the effect of probiotics on the *H. pylori* eradication-induced side effects due to perturbation of gut microbiota. Naturally, the probiotics were tried to overcome this side effects and at the same time to increase the eradication therapy. However, the effects of probiotics are still controversial as the results differ from study to study. Further studies are needed to investigate probiotics' effects on eradication of *H. pylori*.

References

1. Franceschi F, Cazzato A, Nista EC, Scarpellini E, Roccarina D, Gigante G, et al. Role of probiotics in patients with *Helicobacter pylori* infection. Helicobacter. 2007;12(Suppl 2):59–63.
2. Felley C, Michetti P. Probiotics and *Helicobacter pylori*. Best Pract Res Clin Gastroenterol. 2003;17:785–91.
3. Vitor JM, Vale FF. Alternative therapies for *Helicobacter pylori*: probiotics and phytomedicine. FEMS Immunol Med Microbiol. 2011;63:153–64.
4. Patel A, Shah N, Prajapati JB. Clinical application of probiotics in the treatment of *Helicobacter pylori* infection-a brief review. J Microbiol Immunol Infect. 2014;47:429–37.
5. Chen L, Xu W, Lee A, He J, Huang B, Zheng W, et al. The impact of *Helicobacter pylori* infection, eradication therapy and probiotic supplementation on gut microenvironment homeostasis: an open-label, randomized clinical trial. EBioMedicine. 2018;35:87–96.
6. Haller D, Bode C, Hammes WP, Pfeifer AM, Schiffrin EJ, Blum S. Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures. Gut. 2000;47:79–87.
7. Saxelin M, Tynkkynen S, Mattila-Sandholm T, de Vos WM. Probiotic and other functional microbes: from markets to mechanisms. Curr Opin Biotechnol. 2005;16:204–11.
8. Perdigòn G, Medina M, Vintiñi E, Valdez JC. Intestinal pathway of internalisation of lactic acid bacteria and gut mucosal immunostimulation. Int J Immunopathol Pharmacol. 2000;13:141–50.
9. Gill HS. Probiotics to enhance anti-infective defences in the gastrointestinal tract. Best Pract Res Clin Gastroenterol. 2003;17:755–73.
10. Kabir AM, Aiba Y, Takagi A, Kamiya S, Miwa T, Koga Y. Prevention of *Helicobacter pylori* infection by *Lactobacilli* in a gnotobiotic murine model. Gut. 1997;41:49–55.
11. Sgouras D, Maragkoudakis P, Petraki K, Martinez-Gonzalez B, Eriotou E, Michopoulos S, et al. In vitro and in vivo inhibition of *Helicobacter pylori* by *Lactobacillus casei* strain Shirota. Appl Environ Microbiol. 2004;70:518–26.
12. Aiba Y, Suzuki N, Kabir AM, Takagi A, Koga Y. Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a gnotobiotic murine model. Am J Gastroenterol. 1998;93:2097–101.
13. Viljanen M, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T, et al. Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: a double-blind placebo-controlled trial. Allergy. 2005;60:494–500.
14. Yang YJ, Chuang CC, Yang HB, Lu CC, Sheu BS. *Lactobacillus acidophilus* ameliorates *H. pylori*-induced gastric inflammation by inactivating the Smad7 and NFkappaB pathways. BMC Microbiol. 2012;12:38.
15. Lesbros-Pantoflickova D, Corthésy-Theulaz I, Blum AL. *Helicobacter pylori* and probiotics. J Nutr. 2007;137:812s–8s.
16. Servin AL. Antagonistic activities of *lactobacilli* and *Bifidobacteria* against microbial pathogens. FEMS Microbiol Rev. 2004;28:405–40.
17. Jack RW, Tagg JR, Ray B. Bacteriocins of gram-positive bacteria. Microbiol Rev. 1995;59:171–200.
18. Klaenhammer TR. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol Rev. 1993;12:39–85.
19. Nam H, Ha M, Bae O, Lee Y. Effect of *Weissella confusa* strain pl9001 on the adherence and growth of *Helicobacter pylori*. Appl Environ Microbiol. 2002;68:4642–5.
20. Byrd JC, Yunker CK, Xu QS, Sternberg LR, Bresalier RS. Inhibition of gastric mucin synthesis by *Helicobacter pylori*. Gastroenterology. 2000;118:1072–9.
21. Mack DR, Ahrne S, Hyde L, Wei S, Hollingsworth MA. Extracellular MUC3 mucin secretion follows adherence of *Lactobacillus* strains to intestinal epithelial cells in vitro. Gut. 2003;52:827–33.
22. Mattar AF, Teitelbaum DH, Drongowski RA, Yongyi F, Harmon CM, Coran AG. Probiotics up-regulate MUC-2 mucin gene expression in a Caco-2 cell-culture model. Pediatr Surg Int. 2002;18:586–90.
23. Johnson-Henry KC, Mitchell DJ, Avitzur Y, Galindo-Mata E, Jones NL, Sherman PM. Probiotics reduce bacterial colonization and gastric inflammation in *H. pylori*-infected mice. Dig Dis Sci. 2004;49:1095–102.
24. Brzozowski T, Konturek PC, Mierzwa M, Drozdowicz D, Bielanski W, Kwiecien S, et al. Effect of probiotics and triple eradication therapy on the cyclooxygenase (COX)-2 expression, apoptosis, and functional gastric mucosal impairment in *Helicobacter pylori*-infected mongolian gerbils. Helicobacter. 2006;11:10–20.

25. Sgouras DN, Panayotopoulou EG, Martinez-Gonzalez B, Petraki K, Michopoulos S, Mentis A. *Lactobacillus johnsonii* LA1 attenuates *Helicobacter pylori*-associated gastritis and reduces levels of proinflammatory chemokines in c57bl/6 mice. Clin Diagn Lab Immunol. 2005;12:1378–86.
26. Ushiyama A, Tanaka K, Aiba Y, Shiba T, Takagi A, Mine T, et al. *Lactobacillus gasseri* OLL2716 as a probiotic in clarithromycin-resistant *Helicobacter pylori* infection. J Gastroenterol Hepatol. 2003;18:986–91.
27. Chen X, Liu XM, Tian F, Zhang Q, Zhang HP, Zhang H, et al. Antagonistic activities of *Lactobacilli* against *Helicobacter pylori* growth and infection in human gastric epithelial cells. J Food Sci. 2012;77:M9–14.
28. Hwang SW, Kim N, Kim JM, Huh CS, Ahn YT, Park SH, et al. Probiotic suppression of the *H. pylori*-induced responses by conjugated linoleic acids in a gastric epithelial cell line. Prostaglandins Leukot Essent Fatty Acids. 2012;86:225–31.
29. Ojetti V, Bruno G, Ainora ME, Gigante G, Rizzo G, Roccarina D, et al. Impact of *Lactobacillus reuteri* supplementation on anti-*Helicobacter pylori* levofloxacin-based second-line therapy. Gastroenterol Res Pract. 2012;2012:740381.
30. Yoon H, Kim N, Kim JY, Park SY, Park JH, Jung HC, et al. Effects of multistrain probiotic-containing yogurt on second-line triple therapy for *Helicobacter pylori* infection. J Gastroenterol Hepatol. 2011;26:44–8.
31. Kim MN, Kim N, Lee SH, Park YS, Hwang JH, Kim JW, et al. The effects of probiotics on PPI-triple therapy for *Helicobacter pylori* eradication. Helicobacter. 2008;13:261–8.
32. Tursi A, Brandimarte G, Giorgetti GM, Modeo ME. Effect of *Lactobacillus casei* supplementation on the effectiveness and tolerability of a new second-line 10-day quadruple therapy after failure of a first attempt to cure *Helicobacter pylori* infection. Med Sci Monit. 2004;10:Cr662–6.
33. Cremonini F, Di Caro S, Covino M, Armuzzi A, Gabrielli M, Santarelli L, et al. Effect of different probiotic preparations on anti-*Helicobacter pylori* therapy-related side effects: a parallel group, triple blind, placebo-controlled study. Am J Gastroenterol. 2002;97:2744–9.
34. Armuzzi A, Cremonini F, Ojetti V, Bartolozzi F, Canducci F, Candelli M, et al. Effect of *Lactobacillus* GG supplementation on antibiotic-associated gastrointestinal side effects during *Helicobacter pylori* eradication therapy: a pilot study. Digestion. 2001;63:1–7.
35. Canducci F, Armuzzi A, Cremonini F, Cammarota G, Bartolozzi F, Pola P, et al. A lyophilized and inactivated culture of *Lactobacillus acidophilus* increases *Helicobacter pylori* eradication rates. Aliment Pharmacol Ther. 2000;14:1625–9.
36. Francavilla R, Lionetti E, Castellaneta SP, Magistà AM, Maurogiovanni G, Bucci N, et al. Inhibition of *Helicobacter pylori* infection in humans by *Lactobacillus reuteri* ATCC 55730 and effect on eradication therapy: a pilot study. Helicobacter. 2008;13:127–34.
37. Miki K, Urita Y, Ishikawa F, Iino T, Shibahara-Sone H, Akahoshi R, et al. Effect of *Bifidobacterium bifidum* fermented milk on *Helicobacter pylori* and serum pepsinogen levels in humans. J Dairy Sci. 2007;90:2630–40.
38. Wang KY, Li SN, Liu CS, Perng DS, Su YC, Wu DC, et al. Effects of ingesting *Lactobacillus*- and *Bifidobacterium*-containing yogurt in subjects with colonized *Helicobacter pylori*. Am J Clin Nutr. 2004;80:737–41.
39. Pantoflickova D, Corthésy-Theulaz I, Dorta G, Stolte M, Isler P, Rochat F, et al. Favourable effect of regular intake of fermented milk containing *Lactobacillus johnsonii* on *Helicobacter pylori* associated gastritis. Aliment Pharmacol Ther. 2003;18:805–13.
40. Felley CP, Corthésy-Theulaz I, Rivero JL, Sipponen P, Kaufmann M, Bauerfeind P, et al. Favourable effect of an acidified milk (LC-1) on *Helicobacter pylori* gastritis in man. Eur J Gastroenterol Hepatol. 2001;13:25–9.
41. Sheu BS, Wu JJ, Lo CY, Wu HW, Chen JH, Lin YS, et al. Impact of supplement with *Lactobacillus*- and *Bifidobacterium*-containing yogurt on triple therapy for *Helicobacter pylori* eradication. Aliment Pharmacol Ther. 2002;16:1669–75.
42. Mrda Z, Zivanović M, Rasić J, Gajin S, Somer L, Trbojević S, et al. Therapy of *Helicobacter pylori* infection using *lactobacillus acidophilus*. Med Pregl. 1998;51:343–5.
43. He C, Xie Y, Zhu Y, Zhuang K, Huo L, Yu Y, et al. Probiotics modulate gastrointestinal microbiota after *Helicobacter pylori* eradication: a multicenter randomized double-blind placebo-controlled trial. Front Immunol. 2022;13:1033063.
44. Qu P, Liu X, Xia X, Xie X, Luo J, Cheng S, et al. Saccharomyces boulardii allows partial patients to avoid reusing bismuth quadruple for *Helicobacter pylori* rescue therapy: a single-center randomized controlled study. Front Cell Inf Microbiol. 2022;12:903002.
45. Viazis N, Argyriou K, Kotzampassi K, Christodoulou DK, Apostolopoulos P, Georgopoulos SD, et al. A four-probiotics regimen combined with a standard *Helicobacter pylori*-eradication treatment reduces side effects and increases eradication rates. Nutrients. 2022;14:632.
46. Paoluzi OA, Blanco GDV, Visconti E, Coppola M, Fontana C, Favaro M, et al. Low efficacy of levofloxacin-doxycycline-based third-line triple therapy for *Helicobacter pylori* eradication in Italy. World J Gastroenterol. 2015;21:6698.
47. Yang C, Liang L, Lv P, Liu L, Wang S, Wang Z, et al. Effects of non-viable *Lactobacillus reuteri* combining with 14-day standard triple therapy on *Helicobacter pylori* eradication: a randomized double-blind placebo-controlled trial. Helicobacter. 2021;26:e12856.
48. McNicholl AG, Molina-Infante J, Lucendo AJ, Calleja JL, Pérez-Aisa Á, Modolell I, et al. Probiotic supplementation with *Lactobacillus plantarum* and

Pediococcus acidilactici for *Helicobacter pylori* therapy: a randomized, double-blind, placebo-controlled trial. Helicobacter. 2018;23:e12529.

49. He C, Kong F, Chai X, Zou C, Zhu X, Zhao D. Effect of probiotic-assisted eradication of cagA+/vacA s1m1 *Helicobacter pylori* on intestinal flora. BioMed Res Intern. 2022;2022:8607671.
50. Francavilla R, Polimeno L, Demichina A, Maurogiovanni G, Principi B, Scaccianoce G, et al. *Lactobacillus reuteri* strain combination in *Helicobacter pylori* infection: a randomized, double-blind, placebo-controlled study. J Clin Gastroenterol. 2014;48:407–13.
51. Chang YW, Park YM, Oh CH, Oh SJ, Cho J-H, Kim J-W, et al. Effects of probiotics or broccoli supplementation on *Helicobacter pylori* eradication with standard clarithromycin-based triple therapy. Korean J Intern Med. 2020;35:574.
52. Seddik H, Boutallaka H, Elkoti I, Nejjari F, Berraida R, Berrag S, et al. *Saccharomyces boulardii* CNCM I-745 plus sequential therapy for *Helicobacter pylori* infections: a randomized, open-label trial. Eur J Clin Pharmacol. 2019;75:639–45.
53. Lim NR, Choi SY, Chung WC. Probiotic supplementation for treatment of *Helicobacter pylori* infection: a double-blind randomized clinical trial. Korean J Helicobacter Up Gastrointest Res. 2023;23:34–41.
54. Liou JM, Jiang XT, Chen CC, Luo JC, Bair MJ, Chen PY, et al. Second-line levofloxacin-based quadruple therapy versus bismuth-based quadruple therapy for *Helicobacter pylori* eradication and long-term changes to the gut microbiota and antibiotic resistome: a multicentre, open-label, randomised controlled trial. Lancet Gastroenterol Hepatol. 2023;8:228–41.
55. FitzGerald J, Patel S, Eckenberger J, Guillemard E, Veiga P, Schäfer F, et al. Improved gut microbiome recovery following drug therapy is linked to abundance and replication of probiotic strains. Gut Microbes. 2022;14:e20964664.
56. Armuzzi A, Cremonini F, Bartolozzi F, Canducci F, Candelli M, Ojetti V, et al. The effect of oral administration of *Lactobacillus* GG on antibiotic-associated gastrointestinal side-effects during *Helicobacter pylori* eradication therapy. Aliment Pharmacol Ther. 2001;15:163–9.
57. Lionetti E, Miniello VL, Castellaneta SP, Magistà AM, de Canio A, Maurogiovanni G, et al. *Lactobacillus reuteri* therapy to reduce side-effects during anti-*Helicobacter pylori* treatment in children: a randomized placebo controlled trial. Aliment Pharmacol Ther. 2006;24:1461–8.
58. Sýkora J, Valecková K, Amlerová J, Siala K, Dedek P, Watkins S, et al. Effects of a specially designed fermented milk product containing probiotic *Lactobacillus casei* DN-114 001 and the eradication of *H. pylori* in children: a prospective randomized double-blind study. J Clin Gastroenterol. 2005;39:692–8.
59. Michetti P, Dorta G, Wiesel PH, Brassart D, Verdu E, Herranz M, et al. Effect of whey-based culture supernatant of *Lactobacillus acidophilus* (*johnsonii*) La1 on *Helicobacter pylori* infection in humans. Digestion. 1999;60:203–9.
60. Imase K, Tanaka A, Tokunaga K, Sugano H, Ishida H, Takahashi S. *Lactobacillus reuteri* tablets suppress *Helicobacter pylori* infection – a double-blind randomised placebo-controlled cross-over clinical study. Kansenshogaku Zasshi. 2007;81:387–93.
61. Kakiuchi T, Mizoe A, Yamamoto K, Imamura I, Hashiguchi K, Kawakubo H, et al. Effect of probiotics during vonoprazan-containing triple therapy on gut microbiota in *Helicobacter pylori* infection: a randomized controlled trial. Helicobacter. 2020;25:e12690.
62. Makelainen H, Tahvonen R, Salminen S, Ouwehand AC. In vivo safety assessment of two *Bifidobacterium longum* strains. Microbiol Immunol. 2003;47:911–4.
63. Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet. 2008;371:651–9.
64. Salminen MK, Rautelin H, Tynkkynen S, Poussa T, Saxelin M, Valtonen V, et al. *Lactobacillus* bacteremia, species identification, and antimicrobial susceptibility of 85 blood isolates. Clin Infect Dis. 2006;42:e35–44.

51

Treatment Guidelines

Ju Yup Lee

Abstract

More than half of the world's population are infected by *Helicobacter pylori* (*H. pylori*). This infectious disease is now included in the 11th revision of the International Classification of Diseases, which, in principle, recommends that all infected patients should receive treatment. The Kyoto global consensus report emphasized that *H. pylori* gastritis should be considered as the main cause for the development of gastric cancer. Therefore, the scope of indications for eradication treatment has been expanded. *H. pylori* treatment guidelines in South Korea, Japan, and Europe have recently been revised according to data from each of those countries. However, emerging antibiotic resistance is an important barrier to *H. pylori* eradication. The recommended *H. pylori* treatment regimens differ among those three guidelines. In this review, recent guidelines and up-to-date research on *H. pylori* treatment are discussed.

Keywords

Helicobacter pylori · Guideline · Treatment Resistance

51.1 Introduction

Helicobacter pylori (*H. pylori*) infection has little tendency to spontaneous cure, and its eradication is only possible through adequate treatment including antibiotics. However, since the majority of individuals infected with *H. pylori* do not show clear clinical symptoms or develop serious diseases such as peptic ulcer disease, gastric cancer (GC), and gastric mucosa-associated lymphoid tissue (MALT) lymphoma, it is important to set clear criteria for selecting the treatment target population among those infected. However, researchers hold many different views about the *H. pylori* eradication treatment target group, and each country has its own diagnosis and treatment guidelines to provide adequate treatment for *H. pylori* infection, taking into account national and regional features in establishing standard therapy [1–3]. The purpose of this work is to investigate the eradication treatment target population and *H. pylori* treatment strategies, thereby focusing on the recently revised guidelines.

51.2 South Korean Guidelines

In South Korea, after the first publication of consensus about the indications for the treatment of *H. pylori* infection in 1998 [4] by the Korean Society of *Helicobacter* and Upper Gastrointestinal Research (now the Korean College of *Helicobacter* and Upper Gastrointestinal Research), new treatment

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guidelines were released in 2009 based on research and literature [5], and its new revised version was released in 2013 [6].

Most recently, Evidence-Based Guidelines for the Treatment of *Helicobacter pylori* Infection in Korea 2020 were published [1]. This clinical practice guidelines for the treatment of *H. pylori* were updated according to evidence-based medicine from a meta-analysis conducted on a target group, receiving the latest level of eradication therapy. The draft recommendations developed based on the meta-analysis were finalized after an expert consensus on three recommendations regarding the indication for treatment and eight recommendations for the treatment itself. Details are shown in Table 51.1 [1].

Table 51.1 Recommendations for the treatment of *H. pylori*

Category	Statements	Level of evidences	Strength of recommendation
Indications	1. *H. pylori* eradication can be helpful to improve the anemia in subset of adults with unexplained iron deficiency anemia	Very low	Weak
	2. *H. pylori* eradication can be recommended after endoscopic resection for *H. pylori* positive gastric adenoma to prevent metachronous recurrence	Low	Weak
	3. *H. pylori* eradication can be recommended for long-term improvement of dyspeptic symptoms in patients with functional dyspepsia	High	Weak
First-line therapy	4. Standard triple therapy (standard dose PPI, amoxicillin 1 g, and clarithromycin 500 mg twice daily) for 14 days is recommended for first-line regimen	Moderate	Strong
	5. Sequential therapy (standard dose PPI, amoxicillin 1 g twice daily for 5 days followed by standard dose PPI, clarithromycin 500 mg, and metronidazole 500 mg twice daily for 5 days) can be one of first-line therapies for *H. pylori* eradication	High	Strong
	6. Concomitant therapy (standard dose PPI, clarithromycin 500 mg, amoxicillin 1 g, and metronidazole 500 mg twice daily for 10 days) is recommended as a first-line treatment	High	Strong
	7. Clarithromycin resistance test by PCR or sequencing is recommended when a 7-day standard triple therapy is considered as a first-line treatment	Low	Strong
	8. Eradication rates of bismuth quadruple therapy (standard dose PPI twice daily, metronidazole 500 mg three times daily, bismuth 120 mg and tetracycline 500 mg four times daily for 10–14 days) are similar to 14 days standard triple therapy, 10 days concomitant therapy, and 10 days sequential therapy. However, because of its high adverse effects and potential use as second-line therapy, it can be recommended to be used as first-line therapy if other first-line therapy options are not available	Moderate	Weak
Salvage therapy	9. After failure of standard triple therapy, a bismuths quadruple therapy (PPI, bismuth, tetracycline, and metronidazole) for 14 days is recommended as a second-line therapy	High	Strong
	10. After failure of non-bismuth quadruple therapy (sequential or concomitant therapy), a bismuth quadruple therapy is recommended as a second-line therapy	Very low	Strong
	11. After failure of bismuth quadruple therapy as first-line or second-line therapy (after failed standard triple or non-bismuth quadruple therapy), a levofloxacin triple therapy is suggested as a salvage therapy	Very low	Weak

PPI proton pump inhibitor, *PCR* polymerase chain reaction
Adapted from Jung et al. [1]

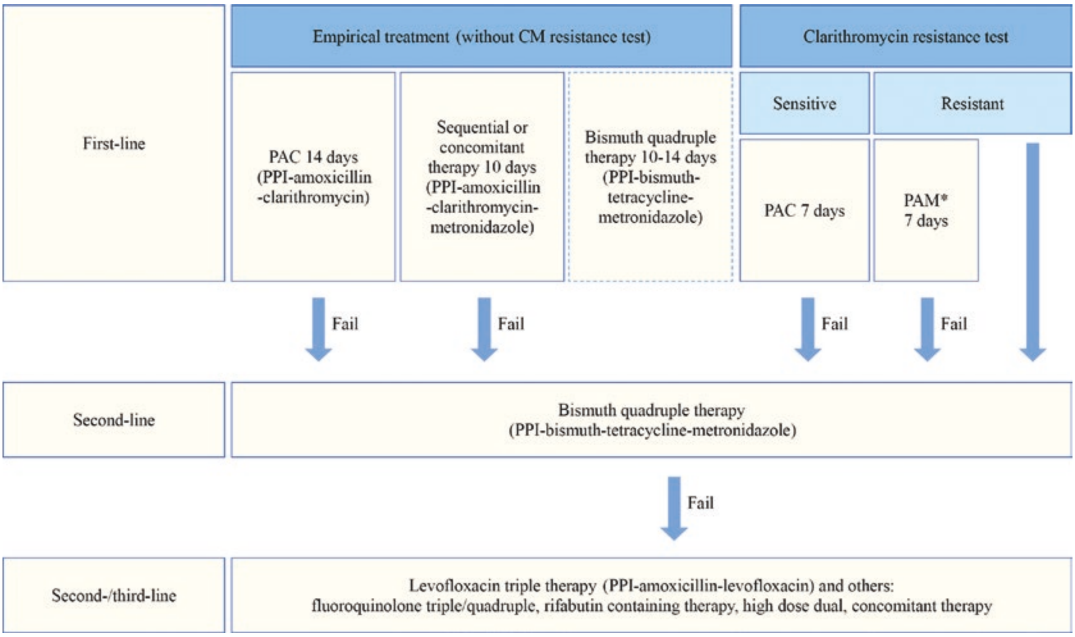

First-line
Empirical treatment (without CM resistance test)
PAC 14 days (PPI-amoxicillin -clarithromycin)
Sequential or concomitant therapy 10 days (PPI-amoxicillin -clarithromycin-metronidazole)
Bismuth quadruple therapy 10-14 days (PPI-bismuth-tetracycline-metronidazole)
Clarithromycin resistance test
Sensitive
Resistant
PAC 7 days
PAM* 7 days
Fail
Fail
Fail
Fail
Second-line
Bismuth quadruple therapy (PPI-bismuth-tetracycline-metronidazole)
Fail
Second-/third-line
Levofloxacin triple therapy (PPI-amoxicillin-levofloxacin) and others: fluoroquinolone triple/quadruple, rifabutin containing therapy, high dose dual, concomitant therapy

Fig. 51.1 Proposed algorithm for *H. pylori* treatment in Korea. Bismuth quadruple therapy as a first-line therapy is dotted because it is less preferred than other regimens. (Adapted from Jung et al. [1]). *CM* clarithromycin, *PPI* proton pump inhibitor. *The PAM regimen consists of PPI, amoxicillin, and metronidazole

In recent years, as the resistance rate of *H. pylori* to clarithromycin has increased, the eradication rate of the existing standard triple therapy hastended to decrease [7–10]. To overcome this, the treatment period has been extended or non-bismuth quadruple therapy such as sequential therapy or concomitant therapy has been introduced [11, 12]. In the case of salvage therapy, it was difficult to select the right randomized controlled trials (RCTs) for each situation due to the diversity of first-line therapy regimens. As a result of meta-analyses of the latest RCTs published, the bismuth quadruple therapy is recommended after standard triple therapy, sequential therapy, or concomitant therapy has failed. If the bismuth quadruple therapy is used as the first-line or salvage therapy, the levofloxacin triple therapy is recommended. However, its effectiveness may be reduced in areas with high resistance to levofloxacin, such as Korea. Recommendation on clarithromycin resistance testing based on polymerase chain reaction (PCR) or sequencing has been made based on the latest literatures [13–15]. Statement regarding tailored therapy will be useful for the use of resistance tests based on PCR or sequencing, which are widely used in clinical practice recently due to the convenience of use (Fig. 51.1).

51.3 Japanese Guidelines

The Japanese Society for *Helicobacter* Research first published “Guidelines for the Management of *H. pylori* Infection in Japan” in 2000 [16] and revised edition in 2010 [17]. The lastest version 2016 edition [2] is the third revision. In Japan, *H. pylori*-infected gastritis has been recognized as an insurance disease since 2013. That is, *H. pylori*-infected gastritis, which has almost the same meaning as *H. pylori* infection, has been recognized as a disease, and diagnosis and treatment of *H. pylori*-related diseases have become possible in real clinical practice. This will affect the treatment and prevention of various diseases associated with *H. pylori* infection, and it is highly likely that a huge contribution will be made to the prevention of GC, which is the big-

Table 51.2 Indications of *Helicobacter pylori* eradication in Japan

Strongly recommended	Probably associated diseases with *H. pylori* infection
• *H. pylori*-related chronic gastritis • Gastric ulcer, duodenal ulcer • Early gastric cancer after endoscopic resection • Gastric MALT lymphoma • Hyperplastic polyp • Functional dyspepsia • Gastroesophageal reflux disease • Idiopathic thrombocytopenic purpura • Iron deficiency anemia	• Chronic urticaria • Cap polyposis • Diffuse large B-cell lymphoma • Rectal MALT lymphoma • Parkinson's syndrome • Alzheimer's disease • Diabetes mellitus

MALT mucosa-associated lymphoid tissue
Adapted from Kato et al. [2] and Kim [18]

gest epidemiological challenge in Japan. Table 51.2 shows the indications for eradication treatment currently presented in the 2016 revised Japanese guidelines for the diagnosis and treatment of *H. pylori* infection.

Japan uses lower-dose antibiotics for eradication treatment when compared to South Korea or Europe. Triple therapy in 7-day b.i.d. (bis in die, twice a day) regimen is recommended as the first-line treatment, consisting of standard-dose proton pump inhibitor (PPI) or potassium ion-competitive acid blocker (P-CAB), 750 mg amoxicillin, and 200 mg (or 400 mg) clarithromycin. As second-line treatment, PPI + 750 mg amoxicillin +250 mg metronidazole in 7-day b.i.d is recommended [2]. No recommendations are made for 14-day regimen of bismuth-containing quadruple therapy as the first- or second-line treatment [19]. The eradication rate in Japan is 75–80% in first-line and 90% in second-line treatments, with the overall eradication rate maintained at 95% or higher via these two therapies [20, 21]. There is no official third-line eradication therapy that is covered by insurance yet in Japan, but the third-line therapy using sitafloxacin is mainly used [22]. Sitafloxacin-based triple therapy has been recommended as a third-line therapy in combination with amoxicillin or metronidazole [23]. Sitafloxacin is a new quinolone antibacterial agent expected to be efficacious due to its low minimum inhibitory concentration (MIC) for *H. pylori*, even for levofloxacin-resistant strains [24]. Sitafloxacin has a good *H. pylori* eradication effect in combination with amoxicillin or metronidazole, even for third-line treatment [25]. The strategy for eradication of *H. pylori* in Japan is shown in Fig. 51.2.

To summarize the characteristics of Japan in terms of eradication treatment, (1) the dose of antibiotics used for eradication treatment, especially clarithromycin, is small, (2) the treatment period is short (7 days), (3) the resistance rate of metronidazole is low, (4) no bismuth quadruple therapy, (5) there is little data on new eradication therapy such as sequential therapy or concomitant therapy, and (6) recently, P-CAB has become available for eradication treatment in addition to PPI.

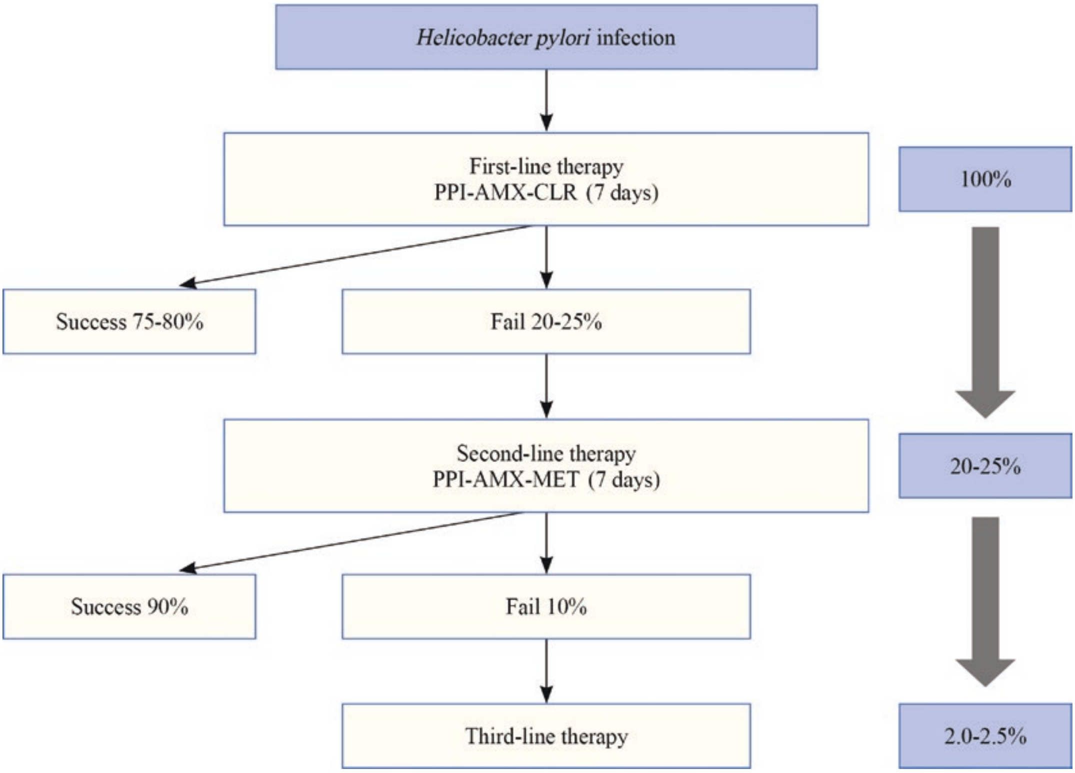


Fig. 51.2 Strategy for *H. pylori* eradication in Japan. (Adapted from Kim [18]). *PPI* proton pump inhibitor, *AMX* amoxicillin, *CLR* clarithromycin, *MET* metronidazole

51.4 European Guidelines (The Maastricht VI/Florence Consensus Report, 2022)

In the present sixth edition of the Maastricht/Florence 2022 Consensus Report [3], key aspects related to the clinical role of *H. pylori* infection were re-evaluated and updated. Forty-one experts from 29 countries representing a global community examined the new data related to *H. pylori* infection in five working groups: (1) indications/associations, (2) diagnosis, (3) treatment, (4) prevention/GC, and (5) *H. pylori* and the gut microbiota. The results of the individual working groups were presented for a final consensus voting that included all participants. Recommendations are provided on the basis of the best available evidence and relevance to the management of *H. pylori* infection in various clinical fields. The main points are summarized as follows [26].

First, *H. pylori* gastritis is an infectious disease. *H. pylori* infects more than half of the world's population. This infectious disease is now included in the 11th revision of the International Classification of Diseases, which, in principle, recommends that all infected patients receive treatment. *H. pylori* causes gastritis, regardless of its symptoms or complications. It is involved in the development of peptic ulcers, dyspepsia, and stomach cancers (adenocarcinoma and MALT lymphoma). It can also be involved in some iron deficiency anemias, vitamin B12 deficiency, and idiopathic thrombocytopenic purpura.

Second, test-and-treat for uninvestigated dyspepsia. Experts are recommending the use of the so-called "test-and-treat" strategy for patients younger than 50 years who have uninvestigated symptoms of dyspepsia and have no alarm symptoms. In dyspeptic patients older than 50 years, particularly patients with coexisting risk factors, upper gastrointestinal endoscopy is recommended.

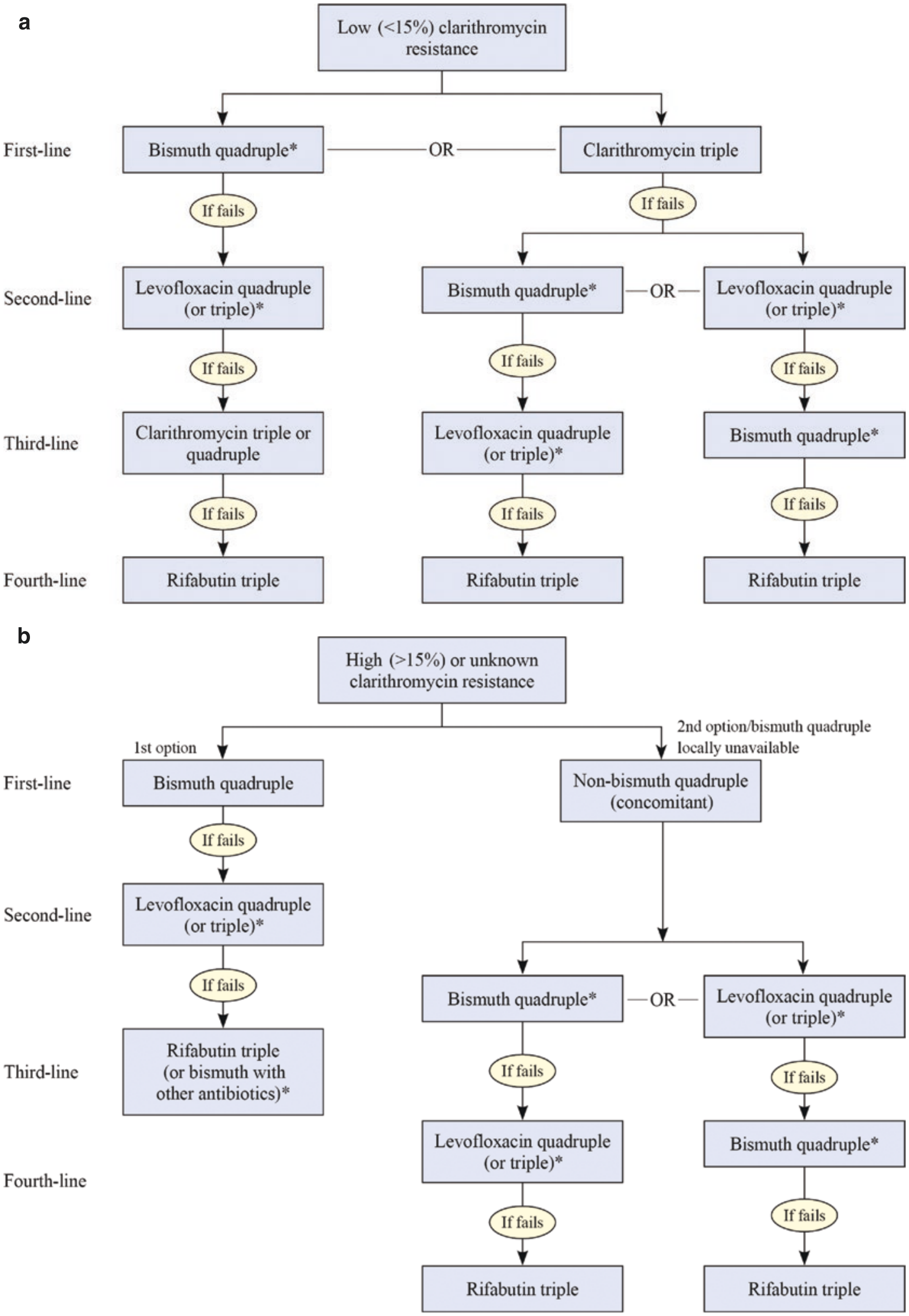
Third, empirical antibiotic treatments should be avoided as much as possible and antibiotic susceptibility is a key consideration. Algorithm for empirical *H. pylori* eradication if individual antibiotic susceptibility testing is not available (Fig. 51.3).

Fourth, the recommendations of Maastricht VI/Florence consensus report have set out the data for deciding on the first-line treatment. If the strain cannot be tested, the treatment strategy depends on the level of resistance to clarithromycin in the region or country in question. Thus, in regions where clarithromycin resistance is greater than 15%, a bismuth-based quadruple therapy is recommended as the first-line treatment (Fig. 51.3).

Fifth, *H. pylori* is the major cause of gastric carcinogenesis. Indeed, *H. pylori* gradually causes severe atrophic gastritis and intestinal metaplasia that may become dysplastic and develop into an adenocarcinoma. However, once the patient has reached the stage of severe atrophic gastritis, the benefits of eradication are uncertain, as lesions are rarely reversible. Therefore, experts stress that although the benefit of eradicating *H. pylori* for preventing cancer applies to all adults, the benefit decreases with age and progression of gastric mucosal lesions. Experts have nonetheless reaffirmed that "test-and-treat" screening campaigns were cost-effective in countries where the incidence of stomach cancer was average or high.

Sixth, the impact of *H. pylori* and its treatments on the microbiota. Pediatricians do not recommend routine eradication of *H. pylori* in children, owing to the impact it may have on the development of certain conditions, especially allergies, via the effect of the antibiotics on the gut microbiota. Probiotics may reduce the side effects of antibiotics (e.g., diarrhea) and thus improve the rates of eradication by promoting better drug compliance.

Fig. 51.3 Algorithm for empirical *Helicobacter pylori* eradication if individual antibiotic susceptibility testing is not available. (Adapted from Malfertheiner et al. [3]). Bismuth quadruple: proton pump inhibitor (PPI), bismuth, tetracycline, and metronidazole. Clarithromycin triple: PPI, clarithromycin and amoxicillin; only use if proven effective locally or if clarithromycin sensitivity is known. (**a**). Non-bismuth quadruple (concomitant): PPI, clarithromycin, amoxicillin, and metronidazole. Levofloxacin quadruple: PPI, levofloxacin, amoxicillin, and bismuth. Levofloxacin triple: the same but without bismuth. In cases of high fluoroquinolone resistance (>15%), the combination of bismuth with other antibiotics, high-dose PPI-amoxicillin dual or rifabutin, may be an option. (**b**). *High-dose PPI or P-CAB (vonoprazan where available) plus amoxicillin may be another option. *P-CAB* potassium ion-competitive acid blocker, *PPI* proton pump inhibitor


a
Low (<15%) clarithromycin resistance
First-line
Bismuth quadruple*
OR
Clarithromycin triple
If fails
Second-line
Levofloxacin quadruple (or triple)*
Bismuth quadruple*
OR
Levofloxacin quadruple (or triple)*
If fails
Third-line
Clarithromycin triple or quadruple
Levofloxacin quadruple (or triple)*
Bismuth quadruple*
If fails
Fourth-line
Rifabutin triple
Rifabutin triple
Rifabutin triple
b
High (>15%) or unknown clarithromycin resistance
1st option
2nd option/bismuth quadruple locally unavailable
First-line
Bismuth quadruple
Non-bismuth quadruple (concomitant)
If fails
Second-line
Levofloxacin quadruple (or triple)*
If fails
Third-line
Rifabutin triple (or bismuth with other antibiotics)*
Bismuth quadruple*
OR
Levofloxacin quadruple (or triple)*
If fails
Fourth-line
Levofloxacin quadruple (or triple)*
Bismuth quadruple*
If fails
Rifabutin triple
Rifabutin triple

51.5 Conclusions

Considering the various guidelines presented above, it can be concluded that gastric ulcer, duodenal ulcer, and gastric MALT lymphoma are classical indications for eradication treatment and the treatment indications are gradually expanding to include function dyspepsia, early GC after endoscopic resection, etc. From the therapeutic perspective, as a countermeasure against the reduction in the eradication rate of standard triple therapy, tailored therapy through antibiotic resistance testing based on PCR is suggested. Continuous research is thus necessary to develop new therapies capable of overcoming antimicrobial resistance issue and to reestablish corresponding treatment guidelines.

References

1. Jung HK, Kang SJ, Lee YC, Yang HJ, Park SY, Shin CM, et al. Evidence-based guidelines for the treatment of *Helicobacter pylori* infection in Korea 2020. Gut Liver. 2021;15:168–95.
2. Kato M, Ota H, Okuda M, Kikuchi S, Satoh K, Shimoyama T, et al. Guidelines for the management of *Helicobacter pylori* infection in Japan: 2016 revised edition. Helicobacter. 2019;24:e12597.
3. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
4. Korean H. Diagnosis and treatment of *Helicobacter pylori* infection in Korea. Korean J Gastroenterol. 1998;32:275–89.
5. Kim N, Kim JJ, Choe YH, Kim HS, Kim JI, Chung IS. Diagnosis and treatment guidelines for *Helicobacter pylori* infection in Korea. Korean J Gastroenterol. 2009;54:269–78.
6. Kim SG, Jung HK, Lee HL, Jang JY, Lee H, Kim CG, et al. Guidelines for the diagnosis and treatment of *Helicobacter pylori* infection in Korea, 2013 revised edition. J Gastroenterol Hepatol. 2014;29:1371–86.
7. Lee JH, Ahn JY, Choi KD, Jung HY, Kim JM, Baik GH, et al. Nationwide antibiotic resistance mapping of *Helicobacter pylori* in Korea: a prospective multicenter study. Helicobacter. 2019;24:e12592.
8. Lee JY, Kim N, Nam RH, In Choi S, Lee JW, Lee DH. Primary and secondary antibiotic resistance of *Helicobacter pylori* in Korea from 2003 to 2018. Helicobacter. 2019;24:e12660.
9. Shin WG, Lee SW, Baik GH, Huh KC, Lee SI, Chung JW, et al. Eradication rates of *Helicobacter pylori* in Korea over the past 10 years and correlation of the amount of antibiotics use: Nationwide survey. Helicobacter. 2016;21:266–78.
10. Kim BJ, Kim HS, Song HJ, Chung IK, Kim GH, Kim BW, et al. Online registry for nationwide database of current trend of *Helicobacter pylori* eradication in Korea: interim analysis. J Korean Med Sci. 2016;31:1246–53.
11. Kim BJ, Lee H, Lee YC, Jeon SW, Kim GH, Kim HS, et al. Ten-day concomitant, 10-day sequential, and 7-day triple therapy as first-line treatment for *Helicobacter pylori* infection: a nationwide randomized trial in Korea. Gut Liver. 2019;13:531–40.
12. Jung YS, Park CH, Park JH, Nam E, Lee HL. Efficacy of *Helicobacter pylori* eradication therapies in Korea: a systematic review and network meta-analysis. Helicobacter. 2017;22:e12389.
13. Lee HJ, Kim JI, Cheung DY, Kim TH, Jun EJ, Oh JH, et al. Eradication of *Helicobacter pylori* according to 23S ribosomal RNA point mutations associated with clarithromycin resistance. J Infect Dis. 2013;208:1123–30.
14. Cho JH, Jeon SR, Kim HG, Jin SY, Park S. Cost-effectiveness of a tailored *Helicobacter pylori* eradication strategy based on the presence of a 23S ribosomal RNA point mutation that causes clarithromycin resistance in Korean patients. J Gastroenterol Hepatol. 2019;34:700–6.
15. Gweon TG, Kim JS, Kim BW. An economic modeling study of *Helicobacter pylori* eradication: comparison of dual priming oligonucleotide-based multiplex polymerase chain reaction and empirical treatment. Gut Liver. 2018;12:648–54.
16. Asaka M, Satoh K, Sugano K, Sugiyama T, Takahashi S, Fukuda Y, et al. Guidelines in the management of *Helicobacter pylori* infection in Japan. Helicobacter. 2001;6:177–86.
17. Asaka M, Kato M, Takahashi S, Fukuda Y, Sugiyama T, Ota H, et al. Guidelines for the management of *Helicobacter pylori* infection in Japan: 2009 revised edition. Helicobacter. 2010;15:1–20.
18. Kim GH. Status of *Helicobacter pylori* eradication in Japan. Korean J Helicobacter Up Gastrointest Res. 2017;17:4.
19. Lee SY. Current progress toward eradicating *Helicobacter pylori* in East Asian countries: differences in the 2013 revised guidelines between China, Japan, and South Korea. World J Gastroenterol. 2014;20:1493–502.
20. Saito Y, Serizawa H, Kato Y, Nakano M, Nakamura M, Saito H, et al. First-line eradication for *Helicobacter pylori*-positive gastritis by esomeprazole-based triple therapy is influenced by CYP2C19 genotype. World J Gastroenterol. 2015;21:13548–54.
21. Asaoka D, Nagahara A, Matsuhisa T, Takahashi S, Tokunaga K, Kawai T, et al. Trends of second-line eradication therapy for *Helicobacter pylori* in Japan: a multicenter study in the Tokyo metropolitan area. Helicobacter. 2013;18:468–72.

22. Cho JH, Jin SY. Current guidelines for *Helicobacter pylori* treatment in East Asia 2022: differences among China, Japan, and South Korea. World J Clin Cases. 2022;10:6349–59.
23. Murakami K, Furuta T, Ando T, Nakajima T, Inui Y, Oshima T, et al. Multi-center randomized controlled study to establish the standard third-line regimen for *Helicobacter pylori* eradication in Japan. J Gastroenterol. 2013;48:1128–35.
24. Sugimoto M, Sahara S, Ichikawa H, Kagami T, Uotani T, Furuta T. High *Helicobacter pylori* cure rate with sitafloxacin-based triple therapy. Aliment Pharmacol Ther. 2015;42:477–83.
25. Nishizawa T, Munkjargal M, Ebinuma H, Toyoshima O, Suzuki H. Sitafloxacin for third-line *Helicobacter pylori* eradication: a systematic review. J Clin Med. 2021;10:2722.
26. Raffier N. The changes made in the new expert consensus on *H. pylori*. Medscape. 2022. https://www.medscape.com/viewarticle/984066. Accessed 15 Nov 2022.

52 Gastric Cancer Screening

Su Youn Nam

Abstract

Gastric cancer (GC) is one of the most common cancers worldwide, and East Asian countries including Korea, Japan, and China have a higher incidence of GC. Although there was no randomized controlled study for the effectiveness of GC screening, Korea and Japan have provided government-supported national screening programs for GC. Several case-control studies and recent cohort studies have reported the effectiveness of radiographic screening for GC in Japan. Japanese guidelines for GC screening recommend that radiographic screening was the only effective method because of a lack of studies using endoscopic screening. From the analysis of Korean national cancer screening data, endoscopy was more effective than radiography for GC screening because the accuracy of endoscopy is much higher than that of radiography. The Korean National Cancer Screening Program has recommended either endoscopy or radiography screening for GC, but the 2015 updated Korean guideline recommends only endoscopy for national cancer screening program. Even if the gold standard of GC screening is upper endoscopy, serum pepsinogen, serum trefoil factor 3, and microRNA has been suggested as alternative test. In this chapter, the accuracy and effectiveness of GC screening by screening modalities and GC screening guidelines of Korea and Japan will be discussed.

Keywords

Gastric cancer · Screening · Mortality Case-control study · Cohort study · Stage shift · Early gastric cancer

52.1 Introduction

Gastric cancer (GC) is the sixth common cancer and ranks as the third leading cause of cancer-related deaths worldwide [1]. East Asian countries including Korea, Japan, and China have a higher incidence of GC than other countries. Korea and Japan have provided government-supported national screening programs for GC. Since 1965, the Japanese government has provided annual radiographic screening. The South Korean government has provided biennial GC screening using endoscopy or radiography to people over 40 years, since 1999 [2]. There was no randomized controlled study for the effectiveness of GC screening. However, several case-control studies and recent cohort studies have reported the effectiveness of radiographic screening for GC in Japan [2, 3]. In 2008, the Japanese guidelines for GC screening through evidence-

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based methods reported that radiographic screening was the only effective method because of a lack of studies using endoscopic screening [3]. From the analysis of Korean national cancer screening data, endoscopy was reported to be more effective than radiography for GC screening because the accuracy of endoscopy was much higher than that of radiography [4]. Before, the 2015 Korean National Cancer Screening Program (NCSP) has recommended either endoscopy or radiography screening for GC but the updated Korean gastric cancer screening guideline in 2015 recommends only esophagogastroduodenoscopy (EGD) for the national cancer screening program based on a meta-analysis for the effect of endoscopic screening on GC mortality [5].

The main modalities for GC screening are EGD and contrast imaging study. EGD allows direct visualization of the entire gastric mucosa and biopsies to confirm the histologic type. Although EGD is more invasive and usually has a higher cost, it is more sensitive for diagnosing gastric lesions. Double-contrast barium radiographs with photofluorography or direct radiography can also identify gastric lesions. However, the rate of the false-negative study was reported up to 50% of cases [6]. In early gastric cancer (EGC), the sensitivity of radiographic study may be as low as 14% [7]. In this chapter, the accuracy and effect of GC screening by screening modalities and gastric cancer screening guidelines of Korea and Japan will be discussed.

52.2 Accuracy of Gastric Cancer Screening Methods

52.2.1 Endoscopic Screening

Sensitivity and specificity of endoscopic screening was constantly high in both Korean and Japanese studies [4, 8]. In a large Korean cohort study using 2,250,392 GC screenees, sensitivity and specificity for 924,822 cases of endoscopic screening were 0.69 (95% confidence interval [CI], 0.67–0.71) and 0.96 (95% CI, 0.96–0.96) [4]. In a Japanese study using 56,676 screenees, sensitivity for 7388 cases of endoscopic screening was 0.89 (95% CI, 0.70–0.98) in the prevalence method and 0.95 (95% CI, 0.84–0.99) in the incidence method and specificity was 0.85 (95% CI, 0.84–0.86) in the prevalence method and 0.89 (95% CI, 0.88–0.89) in the incidence method [8].

52.2.2 Radiographic Screening

Sensitivity of radiographic screening for GC was far different between the Korean and the Japanese study (Fig. 52.1). A large Korean study showed very low sensitivity (0.37) and high specificity (0.96) [4]. Sensitivity from a Costa Rican study by Rosero-Bixby (58%) was better 58% than that of the Korean study [9]. A Japanese study showed higher sensitivity ranging from 0.69 to 0.93 and

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Choi (2012)	1,196	68,853	2,067	1,693,793	0.37 [0.35, 0.38]	0.96 [0.96, 0.96]
Rosero-Rixby (2007)	38	163	27	6,600	0.58 [0.46, 0.71]	0.98 [0.97, 0.98]
Fukao (1992)	356	17,375	158	138,049	0.69 [0.65, 0.73]	0.89 [0.89, 0.89]
Abe (2000)	315	15,684	77	152,417	0.80 [0.76, 0.84]	0.91 [0.91, 0.91]
Ishida (1994)	53	6,703	10	29,055	0.84 [0.73, 0.92]	0.81 [0.81, 0.82]
Hamashima (2013)	48	2,001	6	14,772	0.89 [0.77, 0.96]	0.88 [0.88, 0.89]
Yamamoto (2010)	227	11,114	19	112,137	0.92 [0.88, 0.95]	0.91 [0.91, 0.91]
Higashiyama (2010)	782	39,953	57	391,107	0.93 [0.91, 0.95]	0.91 [0.91, 0.91]

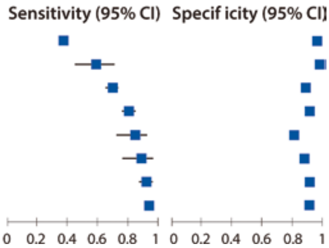

Fig. 52.1 Meta-analysis on the accuracy of radiographic screening. *TP* true-positive, *FP* false-positive, *FN* false-negative, *TN* true-negative, *CI* confidence interval. (Adapted from Park et al. [5])

lower specificity ranging from 0.88 to 0.91 than that of the Korean study [8, 10–13]. Pooled sensitivity of radiographic screening except that of the Korean study was 0.84 [5].

Possible explanations for the different sensitivities are the screening intervals (2 years in Korea vs. 1 year in Japan), the definition of positive findings (only gastric cancer-suspected lesions were regarded as positive findings in the Korean study but all abnormal findings were considered as positive findings in Japanese studies), or the film-reading system (one reader in Korea vs. two well-trained readers in Japan).

52.2.3 Comparison of Sensitivity Between Endoscopic and Radiographic Screening

One Korean and one Japanese study showed the sensitivity of endoscopic and radiographic screening at the same time [4, 8]. A huge Korean cohort study showed higher sensitivity for endoscopic screening compared to radiographic screening (69.0 vs. 36.7%) and similar specificity (96.0 vs. 96.1%) [4]. A Japanese case-control study also showed similar pattern [8].

52.3 Effect of Gastric Cancer Screening on Gastric Cancer Mortality

52.3.1 Endoscopic Screening

Two case-control and two cohort studies evaluated the effect of endoscopic screening on GC mortality in the general population [14–17]. In a case-control study by Hamashima et al., the odds ratio (OR) for endoscopic screening within 36 months of the date of diagnosis was 0.70 [14]. The Korean NCSP report by Cho enrolled a baseline cohort between 2002 and 2003 and investigated GC development up to 2008 and GC death up to 2011 [15]. Endoscopic screening decreased GC mortality (adjusted OR 0.43). A Japanese cohort study by Matsumoto showed that the relative risk (RR) of GC mortality was 0.35 [16]. A Japanese cohort study by Hosokawa demonstrated that the RR for GC death in the screened group was 0.35 [17]. Three Japanese studies and a large Korean study constantly showed that endoscopic screening reduced GC mortality. In a meta-analysis of case-control studies, upper endoscopy significantly reduced GC mortality (pooled OR 0.46; 95% CI, 0.44–0.49), and a meta-analysis of cohort studies also showed significant reduction in GC mortality (pooled OR 0.35; 95% CI, 0.15–0.80) [5].

52.3.2 Radiographic Screening

The effect of radiographic screening on GC mortality was far different among nations. A case-control study by Oshima et al. reported that the OR of GC mortality in the radiographic-screened group was estimated at 0.52 (90% CI, 0.30–0.91) in men and 0.49 (90% CI, 0.24–0.99) in women [18]. A case-control study in Venezuela demonstrated that radiographic screening reduced GC mortality [19]. In a case-control study from Miyagi prefecture in Japan, radiographic screening reduced GC mortality in men but not in women [20]. Another case-control study by Tsubono et al. reported a reduction in mortality of 79% in the radiographic-screened group [21]. However, radiographic screening did not reduce GC mortality in a study by Hamashima et al. [14]. Cho et al. conducted a large-scale case-control study from the Korean NCSP. Radiographic screening showed only a 7% reduction in GC mortality [15].

A population-based cohort study by Inaba et al. reported that radiographic screening did not reduce GC mortality significantly in both men and women [22]. In a prospective cohort study by Mizoue et al., radiographic screening reduced GC mortality in men but not in women [23]. In a population-based prospective cohort study by Lee et al., 40% reduction in GC death was noted in the screened group [24]. Another Japanese cohort study reported that radiographic screening reduced GC mortality [25]. A cohort study from Costa Rica suggested that GC mortality was about twofold higher in unscreened groups than in screened groups [9]. A cohort study by Matusmoto demonstrated that GC mortality was 0.13% in the unscreened group and 0.07% in the radiographic-screened group [16].

Cohort studies from Japan and Costa Rica showed constant reduction of GC mortality in the radiographic-screened group. However, the effect from case-control studies has discrepancy by nations. Japan and Venezuela studies suggested significant reduction of GC mortality, but a huge Korean case-control study showed just 7% reduction in the radiographic-screened group. The main cause of this discrepancy seems to originate from the sensitivity difference of radiographic screening. The sensitivity was 69–83% in the Japanese studies but 37% in the Korean study.

52.4 Guidelines

52.4.1 Updated Korean Guideline 2015

The Korean government has provided a GC screening program for the general population since 1999, even if the effectiveness or harms of GC screening using EGD and radiographic screening had not been fully evaluated. Recently, the Korean multidisciplinary expert committee for developing a GC screening guideline systematically reviewed the evidence regarding the benefits and harms of GC screening and developed an evidence-based clinical guideline [5] (Table 52.1). They found a "low"-level evidence that GC screening using EGD or upper gastrointestinal series could reduce GC mortality in the general population between 40 and 74 years. The benefits of GC screening using EGD were substantially higher than its harms, while the benefits of radiographic screening were moderately higher. Therefore, this guideline recommended biannual EGD for asymptomatic adults from 40 to 75 years (recommendation B). Radiographic screening in asymptomatic adults between 40 and 74 years could be recommended based on clinicians' judgment regarding the patient's risk and the patient's preference (recommendation C). GC screening had insufficient evidence (recommendation I) for adults aged between 75 and 84 years and was not recommended for asymptomatic adults older than 85 years (recommendation D).

52.4.2 Further Supporting Evidence After 2015 Guideline

A case-control study using data from the Korean NSCP for GC (54,418 case and 217,672 matched controls among16 million cancer free adults) showed that the overall OR for dying from GC among ever-screened subjects was 0.79 (95% CI, 0.77–0.81). The ORs of death from GC were 0.53 (95% CI, 0.51–0.56) for upper endoscopy and 0.98 (95% CI, 0.95–1.01) for UGI series [26]. Furthermore, all causes mortality and GC specific morality was higher in the screened group compared to the non-screened group in age older

Table 52.1 Updated Korean gastric cancer screening guideline

			Benefit size		
		Substantial	Moderate	Small	Zero/negative
Evidence level	High	A	B	C	D
	Moderate	A	B	C	D
	Low	B (EGD from 40 to 74 years)	C (radiographic screening from 40 to 74 years)	C	D (asymptomatic adults older than 85 years)
	Very low	I	I	I (adults aged between 75 and 84 years)	I

Recommendation grade: *A* strong recommendations for screening, *B* moderate recommendations for screening, *C* selective recommendation considering clinical decision and screenee's favorites, *D* no recommendation for screening, *I* insufficient evidence to estimate the size of benefit and harm, *EGD* esophagogastroduodenoscopy than 75 years. This study is a formal published article of the gray literature [15], and this content is reflected in the 2015 guidelines.

In a recent study using national cohort between 2004 and 2013, patients with GC who participated in the NCSP at least once (the screening group, n = 116,775) were compared with those who did not participate in the NCSP (the non-screening group, n = 74,927) [27]. The screening group was associated with lower medical care expenses and a better prognosis (age-standardized mortality rate ratio of the screening vs. the non-screening group; 0.62) than the nonscreening group. The NCSP for GC was cost-effective for treatment prognosis. In another recent study, 46,701 GC patients diagnosed in 2008 and 2009 were followed-up until 2019. The survival rate was higher among screened patients (65.8%) than never-screened patients (49.1%). Screened patients were associated with lower risk of death from GC [overall hazard ratio (HR), 0.47; HR in upper endoscopy group, 0.36; HR in UGI series, 0.69] [28]. Screening effect was marked in patients within 2 years prior to cancer diagnosis. The published articles since the 2015 Guidelines reinforce the rationale for the 2015 Guidelines.

52.4.3 Japanese Guideline

The Japanese committee evaluated four methods for GC screening (photofluorography, endoscopy, serum pepsinogen testing, and *H. pylori* antibody testing) and reported the screening guideline 2008. The studies that evaluated mortality reduction from GC included five case-control and two cohort studies for radiographic screening. GC screening using photofluorography was recommended. The other methods were not recommended for population-based screening due to insufficient evidence. However, the revised 2014 Japanese Guidelines approved upper endoscopy for population-based screening, and some provinces have used both endoscopic and radiographic study for GC screening. In Japan, endoscopic GC screening has begun but it presents challenges such as limited regional availability of endoscopists. Japan proposed a GC screening scheme by risk stratification (age, *H. pylori* status, and epidemiologic risk factors) [29] (Fig. 52.2).

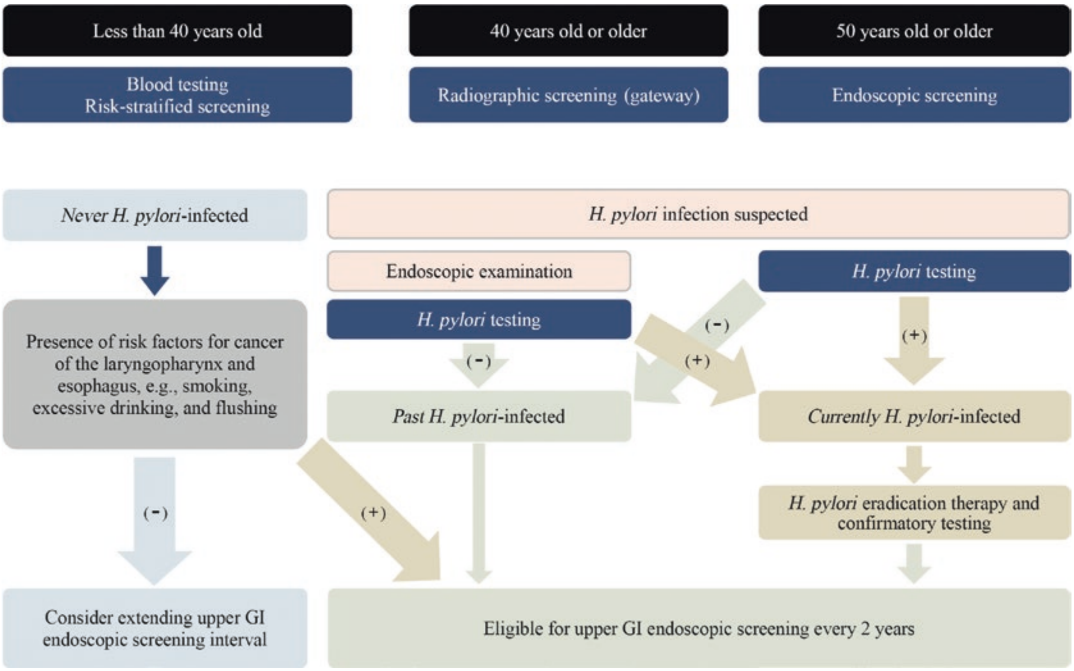
Less than 40 years old
40 years old or older
50 years old or older
Blood testing
Risk-stratified screening
Radiographic screening (gateway)
Endoscopic screening
Never H. pylori-infected
H. pylori infection suspected
Endoscopic examination
H. pylori testing
H. pylori testing
Presence of risk factors for cancer of the laryngopharynx and esophagus, e.g., smoking, excessive drinking, and flushing
(-)
(-)
(+)
(+)
Past H. pylori-infected
Currently H. pylori-infected
H. pylori eradication therapy and confirmatory testing
(-)
(+)
Consider extending upper GI endoscopic screening interval
Eligible for upper GI endoscopic screening every 2 years

Fig. 52.2 A proposed gastric cancer screening scheme in Japan. (Adapted from Mabe et al. [29])

52.5 Serologic Test for Gastric Cancer Screening

52.5.1 Serum Pepsinogen

Low serum pepsinogen (PG) I and low serum PG I/II ratios suggest the presence of atrophic gastritis, a risk factor for gastric cancer. Therefore, this marker has been proposed to identify high-risk individuals [30, 31]. However, in a pooled analysis of 27 population-based screening studies and 15 studies in high-risk groups (300,000 individuals), the pooled sensitivity and specificity of serum PG (PG I level ≤ 70 ng/mL and serum PG I/II ratio < 3) for GC were only 77% and 73% in population-based studies and 57% and 80% in high-risk groups [31].

52.5.2 Serum Trefoil Factor 3

Serum trefoil factor 3 (TFF3) is a stable protein expressed in intestinal goblet cells and gastric intestinal metaplasia. In a Japanese study, the sensitivity and specificity of serum TFF3 for the detection of GC were both 81% [32]. The combination of PG and TFF3 may provide higher sensitivity for GC (sensitivity of 80% in PG + TFF3 test and 67% in PG test) [33]. A Korean case-control study using 281 EGC who underwent endoscopic resection and 708 healthy controls showed that the sensitivity of PG + TFF3 combination for EGC detection was superior (87.5%) to that of TFF3 (80.4%) or PG test alone (39.5%). In the validation cohort, the sensitivity of TFF3 + PG was higher (90.4%) than that of TFF3 (80.0%) or PG test alone (33.3%) [34]. However, accuracy (area under curve, AUC) was modest; AUC is 0.756 in TFF3 + PG combination, 0.651 in TFF3 alone, and 0.633 in PG alone. Prospective studies are needed to investigate the screening values of TFF3 with upper endoscopy.

52.5.3 MicroRNAs

Several microRNAs (miRNAs, e.g., miR-421, miR-18a, and miR-106a) are highly expressed in gastric cancers and are detectable in peripheral blood [35–37]. In high-risk population, serum 12-miRNAs panel showed high accuracy; AUC = 0.93 and AUC = 0.92 in the discovery and verification cohorts, respectively [38]. In the prospective study, sensitivity was 87.0% and specificity was 68.4%. AUC was 0.848, higher than *H. pylori* serology (0.635), PG I/II ratio (0.641), PG index (0.576), and ABC method (0.647). Additional studies are needed to define the role of miRNAs as biomarkers for gastric cancer. Further studies are needed to define the role of miRNAs for use in GC screening.

52.5.4 Multianalyte Blood Tests

In a primitive study, combinations of tumor-specific circulating proteins and mutations in cell-free DNA (CancerSEEK) showed sensitivity of 75% with a high specificity in cancers diagnosed with stage I to III [39]. However, prospective trials in large populations are needed to establish its clinical usefulness and demonstrate its efficacy.

52.6 Conclusion

Korea and Japan, high-prevalence areas of gastric cancer, have provided national screening programs for gastric cancer. Whereas the Japanese government has provided annual radiographic screening, the South Korean government has provided biennial GC screening using endoscopy or radiography to people over 40 years. Even if there was no randomized controlled study for the effectiveness of GC screening, several case-control studies and recent cohort studies have reported the effectiveness of radiographic screening for GC in Japan. However, recent population-based screening studies demonstrated reduced GC death in the endoscopic screening group. Furthermore, endoscopic screening was more sensitive than radiographic screening and constantly reduced GC mortality, whereas the sensitivity of radiographic screening was variable and mortality reduction was also variable by study area. Therefore, the 2015 updated Korean guideline recommends only EGD for GC screening because of the low sensitivity and minimal reduction of GC death of radiographic screening in Korea. Although the revised 2014 Japanese Guidelines approved upper endoscopy for population-based screening, endoscopic screening presents limited availability of endoscopists in Japan. They proposed a GC screening scheme by risk stratification.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209–49.
2. Shen L, Shan YS, Hu HM, Price TJ, Sirohi B, Yeh KH, et al. Management of gastric cancer in Asia: resource-stratified guidelines. Lancet Oncol. 2013;14:e535–47.
3. Hamashima C, Shibuya D, Yamazaki H, Inoue K, Fukao A, Saito H, et al. The Japanese guidelines for gastric cancer screening. Jpn J Clin Oncol. 2008;38:259–67.
4. Choi KS, Jun JK, Park EC, Park S, Jung KW, Han MA, et al. Performance of different gastric cancer screening methods in Korea: a population-based study. PLoS One. 2012;7:e50041.
5. Park HY, Nam SY, Lee SK, Kim SG, Shim KN, Park SM, et al. The Korean guideline for gastric cancer screening. J Korean Med Assoc. 2015;58:373–84.
6. Dooley CP, Larson AW, Stace NH, Renner IG, Valenzuela JE, Eliasoph J, et al. Double-contrast barium meal and upper gastrointestinal endoscopy. A comparative study. Ann Intern Med. 1984;101:538–45.
7. Longo WE, Zucker KA, Zdon MJ, Modlin IM. Detection of early gastric cancer in an aggressive endoscopy unit. Am Surg. 1989;55:100–4.
8. Hamashima C, Okamoto M, Shabana M, Osaki Y, Kishimoto T. Sensitivity of endoscopic screening for gastric cancer by the incidence method. Int J Cancer. 2013;133:653–9.
9. Rosero-Bixby L, Sierra R. X-ray screening seems to reduce gastric cancer mortality by half in a community-controlled trial in Costa Rica. Br J Cancer. 2007;97:837–43.
10. Yamamoto K, Yamazaki H, Kuroda C, Kubo T, Oshima A, Katsuda T, et al. Diagnostic validity of high-density barium sulfate in gastric cancer screening: follow-up of screenees by record linkage with the Osaka cancer registry. J Epidemiol. 2010;20:287–94.
11. Ishida T, Suematsu T, Oobayashi K, Takada Y, Kimura S, Suematsu C. Measurement of accuracy of stomach mass screening by population-based cancer registration. J Gastroenterol Mass Surv. 1994;32:9–16.
12. Fukao A, Hisamichi S, Takano A, Sugahara N. Accuracies of mass screening for gastric cancer: test sensitivity and program sensitivity. J Gastroenterol Mass Surv. 1992;97:59–63.
13. Abe S, Shibuya D, Noguchi T, Shimada T. An estimate of the false-negative rate of mass-screening for gastric carcinoma. J Gastroenterol Mass Surv. 2000;38:475–82.
14. Hamashima C, Ogoshi K, Okamoto M, Shabana M, Kishimoto T, Fukao A. A community-based, case-control study evaluating mortality reduction from gastric cancer by endoscopic screening in Japan. PLoS One. 2013;8:e79088.
15. Cho BR. Evaluation of the validity of current national health screening program and plan to improve the system. Korea Centers for Disease Control and Prevention. 2013. http://www.cdc.go.kr/CDC/notice/CdcKrInfo0201.jsp?menuIds=HOME001-MNU1154-MNU0004-MNU1889&cid=21792. Accessed 30 Oct 2013.
16. Matusmoto S. Effectiveness of gastric endoscopic screening in reducing the mortality of gastric cancer, in comparison with barium x-ray examination and non-examination. J Gastroenterol Cancer Screen. 2010;48:436–41.
17. Hosokawa O, Miyanaga T, Kaizaki Y, Hattori M, Dohden K, Ohta K, et al. Decreased death from gastric cancer by endoscopic screening: association with a population-based cancer registry. Scand J Gastroenterol. 2008;43:1112–5.
18. Oshima A, Hirata N, Ubukata T, Umeda K, Fujimoto I. Evaluation of a mass screening program for stomach cancer with a case-control study design. Int J Cancer. 1986;38:829–33.
19. Pisani P, Oliver WE, Parkin DM, Alvarez N, Vivas J. Case-control study of gastric cancer screening in Venezuela. Br J Cancer. 1994;69:1102–5.
20. Fukao A, Tsubono Y, Tsuji I, Hisamichi S, Sugahara N, Takano A. The evaluation of screening for gastric cancer in Miyagi prefecture, Japan: a population-based case-control study. Int J Cancer. 1995;60:45–8.
21. Tsubono Y, Hisamichi S. Case-control studies of screening for gastric cancer in Japan. J Gastroenterol Mass Surv. 1999;37:182–5.
22. Inaba S, Hirayama H, Nagata C, Kurisu Y, Takatsuka N, Kawakami N, et al. Evaluation of a screening program on reduction of gastric cancer mortality in Japan: preliminary results from a cohort study. Prev Med. 1999;29:102–6.
23. Mizoue T, Yoshimura T, Tokui N, Hoshiyama Y, Yatsuya H, Sakata K, et al. Prospective study of screening for stomach cancer in Japan. Int J Cancer. 2003;106:103–7.
24. Lee KJ, Inoue M, Otani T, Iwasaki M, Sasazuki S, Tsugane S, JPHC Study Group. Gastric cancer screening and subsequent risk of gastric cancer: a large-scale population-based cohort study, with a 13-year follow-up in Japan. Int J Cancer. 2006;118:2315–21.
25. Miyamoto A, Kuriyama S, Nishino Y, Tsubono Y, Nakaya N, Ohmori K, et al. Lower risk of death from gastric cancer among participants of gastric cancer screening in Japan: a population-based cohort study. Prev Med. 2007;44:12–9.

26. Jun JK, Choi KS, Lee HY, Suh M, Park B, Song SH, et al. Effectiveness of the Korean National Cancer Screening Program in reducing gastric cancer mortality. Gastroenterology. 2017;152:1319–28.
27. Suh YS, Lee J, Woo H, Shin D, Kong SH, Lee HJ, et al. National cancer screening program for gastric cancer in Korea: Nationwide treatment benefit and cost. Cancer. 2020;126:1929–39.
28. Luu XQ, Lee K, Jun JK, Suh M, Jung KW, Choi KS. Effect of gastric cancer screening on long-term survival of gastric cancer patients: results of Korean national cancer screening program. J Gastroenterol. 2022;57:464–75.
29. Mabe K, Inoue K, Kamada T, Kato K, Kato M, Haruma K. Endoscopic screening for gastric cancer in Japan: current status and future perspectives. Dig Endosc. 2022;34:412–9.
30. Yoshida T, Kato J, Inoue I, Yoshimura N, Deguchi H, Mukoubayashi C, et al. Cancer development based on chronic active gastritis and resulting gastric atrophy as assessed by serum levels of pepsinogen and *Helicobacter pylori* antibody titer. Int J Cancer. 2014;134:1445–57.
31. Miki K. Gastric cancer screening using the serum pepsinogen test method. Gastric Cancer. 2006;9:245–53.
32. Aikou S, Ohmoto Y, Gunji T, Matsuhashi N, Ohtsu H, Miura H, et al. Tests for serum levels of trefoil factor family proteins can improve gastric cancer screening. Gastroenterology. 2011;141:837–45.
33. Kaise M, Miwa J, Tashiro J, Ohmoto Y, Morimoto S, Kato M, et al. The combination of serum trefoil factor 3 and pepsinogen testing is a valid nonendoscopic biomarker for predicting the presence of gastric cancer: a new marker for gastric cancer risk. J Gastroenterol. 2011;46:736–45.
34. Lee HS, Jeon SW, Nomura S, Seto Y, Kwon YH, Nam SY, et al. Screening biomarker as an alternative to endoscopy for the detection of early gastric cancer: the combination of serum trefoil factor family 3 and pepsinogen. Gastroenterol Res Pract. 2018;2018:1024074.
35. Zhou H, Guo JM, Lou YR, Zhang XJ, Zhong FD, Jiang Z, et al. Detection of circulating tumor cells in peripheral blood from patients with gastric cancer using microRNA as a marker. J Mol Med (Berl). 2010;88:709–17.
36. Cui L, Zhang X, Ye G, Zheng T, Song H, Deng H, et al. Gastric juice MicroRNAs as potential biomarkers for the screening of gastric cancer. Cancer. 2013;119:1618–26.
37. Su ZX, Zhao J, Rong ZH, Wu YG, Geng WM, Qin CK. Diagnostic and prognostic value of circulating miR-18a in the plasma of patients with gastric cancer. Tumour Biol. 2014;35:12119–25.
38. So JBY, Kapoor R, Zhu F, Koh C, Zhou L, Zou R, et al. Development and validation of a serum microRNA biomarker panel for detecting gastric cancer in a high-risk population. Gut. 2021;70:829–37.
39. Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science. 2018;359:926–30.

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Recrudescence and Reinfection After *H. pylori* Eradication Treatment

Nayoung Kim

Abstract

Helicobacter pylori (*H. pylori*) recurrence can be classified into two classes: recrudescence and reinfection. Recrudescence is a recurrence of the same infection after antimicrobial treatment, which occurs as first-year relapses. On the other hand, reinfection is defined as a state wherein a patient becomes infected with a new *H. pylori* strain after the patient went through successful eradication therapy. A DNA fingerprinting technique is utilized to distinguish between recrudescence and reinfection. However, this technique cannot be done for every single analysis, so numerous researches define reinfection as a *H. pylori*-positive state after 12 months of *H. pylori*-negative condition during a monitoring period once an eradication treatment is over. In terms of *H. pylori* reinfection, the worldwide annual reinfection rate is reported to be 0–23.4% on adults and 1.9–9.6% on pediatrics, but the rates are less than 1% in the developed countries, while they are high among developing nations. The risk factors of this recurrence were noncompliance to the initial therapy, presence of children in the household, male, and low income. Thus, the reinfection rate of *H. pylori* will further decrease if socioeconomic condition and public hygiene continue to ameliorate.

Keywords

Recrudescence · Reinfection · *Helicobacter pylori* · Risk factors

53.1 Introduction

Helicobacter pylori (*H. pylori*) recurrence is an important factor to determine the efficacy of antimicrobial treatment, because if there is a high conversion ratio of patients who were previously determined to be *H. pylori*-negative into *H. pylori*-positive, then applied antimicrobial treatment would be useless. Patients with short-term recurrence suffer from the risk of recurrence of these diseases [1]. The economic pressure, psychological burden, and potential adverse drug reactions have increased recurrence rate dramatically [1]. Gisbert et al. suggested that the cumulative annual recurrence rate after *H. pylori* eradication was 5.3% after 1 year, 6.8% after 2 years, 7% after 3 years, 7.6% after 4 years, and 9.3% after 5 years [2]. This study suggested that recurrence decreased with time and declined sharply after the first year [2]. Kim et al. performed a 2-year follow-up of patients with *H.*

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pylori eradication [3]. They found that, regardless of first-line or second-line therapy, the recurrence rate in the first year was significantly different (9.3 vs. 4.5%) and the recurrence rate in the second year was similar (2.0 vs. 2.9%) [3].

H. pylori recurrence can be classified into two classes: recrudescence and reinfection [3]. Recrudescence is a recurrence of the same infection after antimicrobial treatment, which usually occurs as first-year relapses. On the other hand, reinfection is defined as a state wherein a patient becomes infected with a new *H. pylori* strain after the patient went through successful eradication therapy [4]. Recrudescence rather than reinfection is likely to be responsible for the most recurrent cases because the recurrences decrease with time, declining sharply after the first year, and identified strains (before and after therapy) were usually genetically identical [5]. For the recurrence-subtype classification, the DNA fingerprinting technique is utilized to distinguish between recrudescence and reinfection. However, this technique cannot be done for every single analysis, so numerous researches define reinfection as an *H. pylori*-positive state after a patient has maintained 12 months of *H. pylori*-negative condition during the monitoring period, since the end of successful eradication treatment [4, 6, 7]. According to the previous research, reinfection rates of adults and pediatrics before the year 2000 were 0–100% and 2.0–13.5%, respectively [4]. After the year 2000, reported "true" reinfection rates in adults generally varied from 0 to 23.4% [5]. The annual "true" reinfection rates were much lower than the reported annual recurrence rates within the first years after eradication [8]. However, the reinfection rates of adults and pediatrics were both below 2% in the developed nations, while they were both higher than 10% in the developing countries [4]. Thus, the research shows that the results are different based on nations and time period [4]. This chapter is going to discuss on the mechanism of *H. pylori* reinfection and how *H. pylori* recurrence rates of adults and pediatrics are different among nations.

53.2 Systematic Review with Meta-Analysis: The Global Recurrence Rate of *H. pylori*

Many studies used reinfection and recurrence interchangeably, because only the minority of studies provided data needed for criteria. Recently, there was a nice systematic review with meta-analysis regarding the global recurrence rate of *H. pylori* [9]. They analyzed total of 132 studies (53,934 patient-years) [9]. Each study was weighted according to the duration of patient-years. The global annual recurrence, reinfection, and recrudescence rate of *H. pylori* were 4.3% (95% confidence interval [CI], 4–5), 3.1% (95% CI, 2–5) and 2.2% (95% CI, 1–3), respectively [9]. The *H. pylori* recurrence rate generally relates to the local sanitary conditions. That is, it was inversely related to the human development index (HDI) (i.e., 3.1% [95% CI, 2–4], 6.2% [95% CI, 4–8] and 10.9% [95% CI, 6–18] in countries with a very high, high, medium, or low HDI) ($p < 0.01$) and directly related to *H. pylori* prevalence (10.9% [95% CI, 7–16], 3.7% [95% CI, 3–5], 3.4% [95% CI, 2–5], and 1.6% [95% CI, 0.5–3] in countries with a very high, high, medium, or low local *H. pylori* prevalence) ($p < 0.01$) [9] (Fig. 53.1). Global recurrence rates remained relatively stable between the years 1990s, 2000s, and 2010s but varied across different regions ($p < 0.05$) [9]. They suggested that the *H. pylori* recurrence remains a problem closely associated with socioeconomic and sanitary conditions.

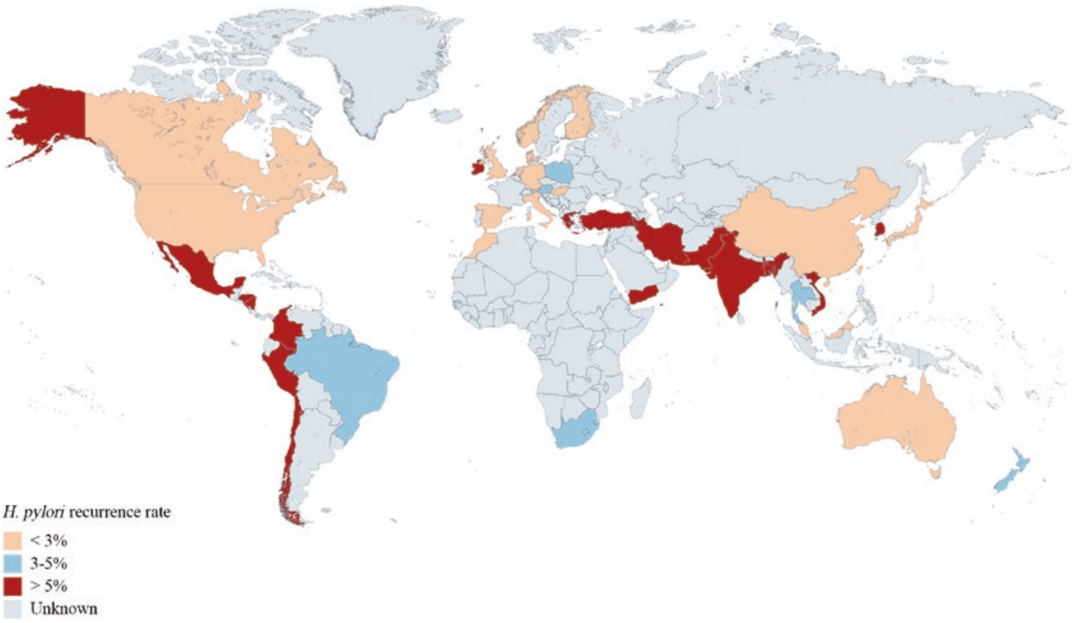

Fig. 53.1 Recurrence prevalence of *Helicobacter pylori* in worldwide population. (Adapted from Hu et al. [9])

53.3 Recrudescence After *H. pylori* Eradication Therapy

Recrudescence is a recurrence of the same infection after antimicrobial treatment, which usually occurs as first-year relapses [10]. This arbitrary definition was confirmed by the report of Take et al. which has been performed among 1609 patients with a mean follow-up of 4.7 years [11]. They compared the strains by the random amplification of polymorphic DNA fingerprinting and showed that during the first year after the successful treatment, six of the 10 strain couples were genetically identical, while none of the four strain couples tested at a later follow-up were identical [11]. This study confirmed that first-year relapses are more likely to be a recurrence of the same infection. Nevertheless, such a recurrence may occur at various rates. In Morocco, the relapse rate was 1 of 239 at 6 months [11], while in Latin America, the 1-year relapse rate was 125 of 1091 (11.5%) patients [12].

The reasons of recrudescence are noncompliance to the initial therapy, presence of children in the household [12], the low efficacy of used antibiotics, and the inaccuracy of *H. pylori* diagnosis 4 weeks after eradication therapy [13]. In other words, recrudescence is highly possible when *H. pylori* eradication was not successful due to the low efficacy of antibiotics, and proper diagnostic method after *H. pylori* eradication is important [13]. For example, there is a high possibility of a false-negative result if the rapid urease test is conducted on patients with either atrophic gastritis or intestinal metaplasia, so conducting modified Giemsa stain histology from the corpus of the stomach gives more accurate result [14–16]. Like this, the false-negative rate of diagnoses after *H. pylori* eradication increases mainly because the sensitivity of *H. pylori* infection diagnoses is not highly enough due to *H. pylori* reduction after the eradication therapy. Especially, *H. pylori* converts itself from spiral bacilli into cocci to survive when it is exposed to antibiotics.

H. pylori in coccus form will not activate its metabolism until its surrounding environment gets better to return itself to bacillus form, and *H. pylori* will not be detected once it is in the coccus form. Thus, administering antibiotics with high efficacy for enough periods and conducting precise *H. pylori* diagnostic methods are necessary to reduce recrudescence. The *H. pylori* recrudescence rates in Korea after administering triple therapy (standard dose of proton pump inhibitor, 1 g of amoxicillin and 500 mg of clarithromycin) as twice a day for 7 days were low such as 3.49% (3/86) in the year 2008 [17] and 4.9% (17/348) in the year 2013 [6], based on the results of *Campylobacter*-like organism (CLO) test, Giemsa histology, and *H. pylori* culture tests from the antrum and corpus.

53.4 Reinfection After *H. pylori* Eradication Therapy

The *H. pylori* infection rate tends to be low in the developed countries and high in the developing countries, and the rate is different based on age, region, and ethnicity. Developed countries, such as the USA or Europe, show the *H. pylori*-positive rate as 10–15% at the age of 3–5, and the rate increases as the age increases. In comparison, the *H. pylori*-positive rate in the developing countries, like Saudi Arabia, was already 40–60% before the age of 10 [18]. Also, the rate among South American nations is currently reported to be 75–83% [19]. *H. pylori* reinfection rates are different among countries by up to 23% [2, 3, 6, 17, 20–27] (Table 53.1), and these rates show similarity with *H. pylori* infection rate, which is low among the developed countries and high among the developing countries [4] (Fig. 53.2). In other words, the reinfection rate on adults and pediatrics before 5 years of age was 0.5–2% in the developed nations [28–33], while the rate under the same conditions was 23.4% in the developing countries [25]. The similarity of low infection and reinfection rates is attributed to high socioeconomic condition and good public hygiene [21]. This situation can be referred to the researches on Mexico and Peru, where *H. pylori*-positive rates were high previously. According to the researches about the *H. pylori* reinfection rate of Mexico and Peru in 2003, their rates were 3.2% and 7.6%, respectively [26, 34]. These values indicate that the socioeconomic and public hygiene conditions of those two nations have improved. Similarly, the *H. pylori* reinfection rate reduction can be seen in Korea, too. According to Kim et al., the *H. pylori* reinfection rate in South Korea was 13% in the year 1998 [35], but the rate was reduced up to 3.51%, after an average of 37.1 months (18–95 months) of follow-up on 331 patients who underwent standard triple therapy [6].

Table 53.1 Reinfection rates of various countries after successful *H. pylori* eradication

Author	Year [reference]	Population	Country	Annual reinfection rate (%)
Rollan et al.	2000 [20]	Adults	Netherlands	1.49
Hildebrand et al.	2001 [21]	Adults	Bangladesh	23.4
Adachi et al.	2002 [22]	Adults	Japan	1.23
Seo et al.	2002 [23]	Adults	Japan	0.00
Gisbert et al.	2006 [2]	Adults	Spain	1.04
Lee et al.	2008 [17]	Adults	Korea	2.94
Kim et al.	2013 [6]	Adults	Korea	3.51
Kim et al.	2014 [3]	Adults	Korea	Within 2 years: 9.3
				After 2 years: 2.0
Bruce et al.	2015 [24]	Adults	Alaska, USA	Cumulative rate within 2 years: 16.1
Kato et al.	1998 [25]	Children	Japan	5.36
Leal-Herrera et al.	2003 [26]	Children	Mexico	9.55
Farrell et al.	2004 [27]	Children	UK	1.91

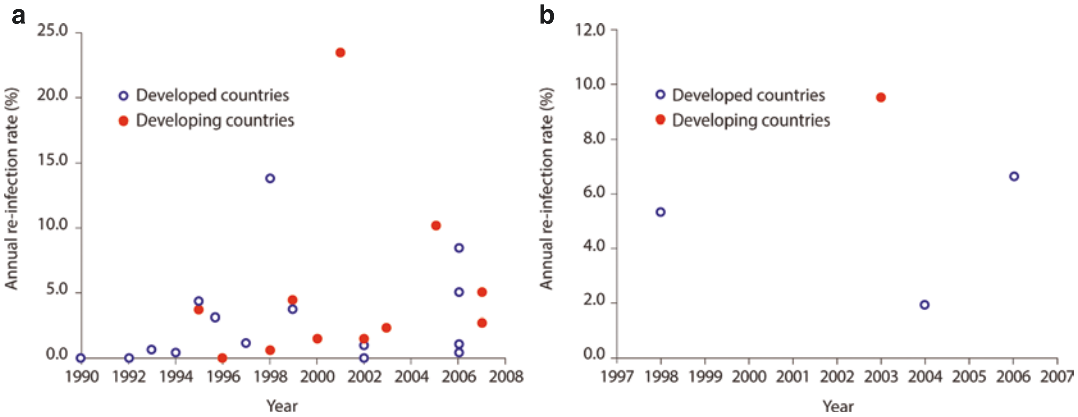

Fig. 53.2 Reinfection distribution of adults (**a**) and pediatrics (**b**) in the developed countries and in the developing countries. (Adapted from Zhang et al. [4])

53.5 Risk Factors of Reinfection After *H. pylori* Eradication Therapy

Recently, Bruce et al. performed an interesting study to determine rates of reinfection in three groups followed for 2 years after successful treatment in 229 persons: American Indian/Alaska Native (AI/AN) persons living in urban (group 1) and rural (group 2) communities and urban Alaska non-native persons (group 3) [24]. *H. pylori* reinfection occurred in 36 persons; cumulative reinfection rates were 14.5%, 22.1%, and 12.0% for groups 1, 2, and 3, respectively [24]. Study participants who became reinfected were more likely to have peptic ulcer disease ($p = 0.02$), low education level ($p = 0.04$), or a higher proportion of household members infected with *H. pylori* compared to participants who did not become reinfected ($p = 0.03$) [24]. In addition, in South Korea, the odds ratio (OR) of the reinfection rate in men was 2.28 (95% CI, 1.05–5.00; $p = 0.037$), which is higher than that in women, and the OR of low income (≤$5000) was 3.54 (95% CI, 1.05–5.00; $p = 0.038$), which was also higher than high income (>$5000) [6], based on a multivariate analysis on factors that affect *H. pylori* reinfection in South Korea, so the risk factors of reinfection are the same as the risk factors for *H. pylori* infection [36–39]. These data imply that the reduced annual *H. pylori* reinfection rate (3.51%) in South Korea is a true reinfection, instead of recrudescence.

53.6 Conclusions

H. pylori recurrence can be classified into two classes: recrudescence and reinfection. Recrudescence is a recurrence of the same infection after antimicrobial treatment, which usually occurs as first-year relapses. On the other hand, reinfection is defined as a patient being infected with a new *H. pylori* strain after the patient went through successful eradication therapy. There is a similarity between infection and reinfection rates of *H. pylori*, which is attributed to socioeconomic condition and public hygiene. Annual *H. pylori* reinfection rates based on worldwide reports are 0–23.4% for adults and 1.9–9.6% on pediatrics and less than 1% in the developed countries and high in the developing countries. This suggests that there is no need to worry about *H. pylori* reinfection in the developed countries, while proper hygiene management after eradication therapy is necessary among the developing nations.

References

1. Sun Y, Zhang J. *Helicobacter pylori* recrudescence and its influencing factors. J Cell Mol Med. 2019;23:7919–25.
2. Gisbert JP, Luna M, Gómez B, Herrerías JM, Monés J, Castro-Fernández M, et al. Recurrence of *Helicobacter pylori* infection after several eradication therapies: long-term follow-up of 1000 patients. Aliment Pharmacol Ther. 2006;23:713–9.
3. Kim SY, Hyun JJ, Jung SW, Koo JS, Yim HJ, Lee SW. *Helicobacter pylori* recurrence after first- and second-line eradication therapy in Korea: the problem of recrudescence or reinfection. Helicobacter. 2014;19:202–6.
4. Zhang YY, Xia HH, Zhuang ZH, Zhong J. Review article 'true' re-infection of *Helicobacter pylori* after successful eradication—worldwide annual rates, risk factors and clinical implications. Ailment Phamacol Ther. 2009;29:145–60.
5. Kayali S, Manfredi M, Gaiani F, Bianchi L, Bizzarri B, Leandro G, et al. *Helicobacter pylori*, ttransmission routes and recurrence of infection: state of the art. Acta Biomed. 2018;89 Suppl 8:72–6.
6. Kim MS, Kim N, Kim SE, Jo HJ, Shin CM, Lee SH, et al. Long-term follow-up *Helicobacter pylori* reinfection rate and its associated factors in Korea. Helicobacter. 2013;18:135–42.
7. Silva FM, Navarro-Rodriguez T, Barbuti RC, Mattar R, Hashimoto CL, Eisig JN. *Helicobacter pylori* reinfection in Brazilian patients with peptic ulcer disease: a 5-year follow-up. Helicobacter. 2010;15:46–52.
8. Sachs G, Scott DR. *H. pylori*: eradication or preservation? F1000 Med Rep. 2012;4:7.
9. Hu Y, Wan JH, Li XY, Zhu Y, Graham DY, Lu NH. Systematic review with meta-analysis: the global recurrence rate of *Helicobacter pylori*. Aliment Pharmacol Ther. 2017;46:773–9.
10. Calvet X, Ramírez Lázaro MJ, Lehours P, Mégraud F. Diagnosis and epidemiology of *Helicobacter pylori* infection. Helicobacter. 2013;18 Suppl 1:5–11.
11. Take S, Mizuno M, Ishiki K, Imada T, Okuno T, Yoshida T, et al. Reinfection rate of *Helicobacter pylori* after eradication treatment: a long-term prospective study in Japan. J Gastroenterol. 2012;47:641–6.
12. Morgan DR, Torres J, Sexton R, Herrero R, Salazar-Martinez E, Greenberg ER, et al. Risk of recurrent *Helicobacter pylori* infection 1 year after initial eradication therapy in 7 Latin American communities. JAMA. 2013;309:578–86.
13. Xia HH, Talley NJ, Keane CT, O'Morain CA. Recurrence of *Helicobacter pylori* infection after successful eradication: nature, possible causes and potential preventive strategies. Dig Dis Sci. 1997;42:1821–34.
14. Kang HY, Kim N, Park YS, Hwang JH, Kim JW, Jeong SH, et al. Progression of atrophic gastritis and intestinal metaplasia driving *Helicobacter pylori* out of the gastric mucosa. Dig Dis Sci. 2006;51:2310–5.
15. Yoo JY, Kim N, Park YS, Hwang JH, Kim JW, Jeong SH, et al. Detection rate of *Helicobacter pylori* against a background of atrophic gastritis and/or intestinal metaplasia. J Clin Gastroenterol. 2007;41:751–5.
16. Shin CM, Kim N, Lee HS, Lee HE, Lee SH, Park YS, et al. Validation of diagnostic tests for *Helicobacter pylori* with regard to grade of atrophic gastritis and/or intestinal metaplasia. Helicobacter. 2009;14:512–9.
17. Lee JH, Kim N, Chung JI, Kang KP, Lee SH, Park YS, et al. Long-term follow up of *Helicobacter pylori* IgG serology after eradication and reinfection rate of *H. pylori* in South Korea. Helicobacter. 2008;13:288–94.
18. Klein PD, Graham DY, Gaillour A, Opekun AR, Smith EO. Water source as risk factor for *Helicobacter pylori* infection in Peruvian children. Gastrointestinal Physiology Working Group. Lancet. 1991;337:1503–6.
19. al-Moagel MA, Evans DG, Abdulghani ME, Adam E, Evans DJ Jr, Malaty HM, et al. Prevalence of *Helicobacter* (formerly *Campylobacter*) *pylori* infection in Saudi Arabia, and comparison of those with and without upper gastrointestinal symptoms. Am J Gastroenterol. 1990;85:944–8.
20. Rollan A, Giancaspero R, Fuster F, Acevedo C, Figueroa C, Hola K, et al. The long-term reinfection rate and the course of duodenal ulcer disease after eradication of *Helicobacter pylori* in a developing country. Am J Gastroenterol. 2000;95:50–6.
21. Hildebrand P, Bardhan P, Rossi L, Parvin S, Rahman A, Arefin MS, et al. Recrudescence and reinfection with *Helicobacter pylori* after eradication therapy in Bangladeshi adults. Gastroenterology. 2001;121:792–8.
22. Adachi M, Mizuno M, Yokota K, Miyoshi M, Nagahara Y, Maga T, et al. Reinfection rate following effective therapy against *Helicobacter pylori* infection in Japan. J Gastroenterol Hepatol. 2002;17:27–31.
23. Seo M, Okada M, Shirotani T, Nishimura H, Maeda K, Aoyagi K, et al. Recurrence of *Helicobacter pylori* infection and the long-term outcome of peptic ulcer after successful eradication in Japan. J Clin Gastroenterol. 2002;34:129–34.
24. Bruce MG, Bruden DL, Morris JM, Reasonover AL, Sacco F, Hurlburt D, et al. Reinfection after successful eradication of *Helicobacter pylori* in three different populations in Alaska. Epidemiol Infect. 2015;143:1236–46.
25. Kato S, Abukawa D, Furuyama N, Iinuma K. *Helicobacter pylori* reinfection rates in children after eradication therapy. J Pediatr Gastroenterol Nutr. 1998;27:543–6.
26. Leal-Herrera Y, Torres J, Monath TP, Ramos I, Gomez A, Madrazo-de la Garza A, et al. High rates of recurrence and of transient reinfections of *Helicobacter pylori* in a population with high prevalence of infection. Am J Gastroenterol. 2003;98:2395–402.
27. Farrell S, Milliken I, Doherty GM, Murphy JL, Wootton SA, McCallion WA. Total family unit *Helicobacter pylori* eradication and pediatric re-infection rates. Helicobacter. 2004;9:285–8.

28. Bell GD, Powell KU. *Helicobacter pylori* reinfection after apparent eradication-the Ipswich experience. Scand J Gastroenterol. 1996;31 Suppl 215:96–104.
29. van der Hulst RW, Rauws EA, Köycü B, Keller JJ, ten Kate FJ, Dankert J, et al. *Helicobacter pylori* reinfection is virtually absent after successful eradication. J Infect Dis. 1997;176:196–200.
30. Rowland M, Kumar D, Daly L, O'Connor P, Vaughan D, Drumm B. Low rates of *Helicobacter pylori* reinfection in children. Gastroenterology. 1999;117:336–41.
31. Borody TJ, Andrews P, Mancuso N, McCauley D, Jankiewicz E, Ferch N, et al. *Helicobacter pylori* reinfection rate, in patients with cured duodenal ulcer. Am J Gastroenterol. 1994;89:529–32.
32. Seppälä K, Färkkilä M, Nuutinen H, Hakala K, Väänänen H, Rautelin H, et al. Triple therapy of *Helicobacter pylori* infection in peptic ulcer. A 12-month follow-up study of 93 patients. Scand J Gastroenterol. 1992;27:973–6.
33. Parsonnet J. The incidence of *Helicobacter pylori* infection. Aliment Pharmacol Ther. 1995;9 Suppl 2:45–52.
34. Soto G, Bautista CT, Roth DE, Gilman RH, Velapatiño B, Ogura M, et al. *Helicobacter pylori* infection is common in Peruvian adults after antibiotic eradication therapy. J Infect Dis. 2003;188:1263–75.
35. Kim N, Lim SH, Lee KH, Jung HC, Song IS, Kim CY, et al. *Helicobacter pylori* reinfection rate and duodenal ulcer recurrence in Korea. J Clin Gastroenterol. 1998;27:321–6.
36. Kim N, Kim JG, Kim JH, Kim HY, Kim SW, Kim JJ, et al. Risk factors of *Helicobacter pylori* infection in asymptomatic Korean population. Korean J Med. 2000;59:376–87.
37. Yim JY, Kim N, Choi SH, Kim YS, Cho KR, Kim SS, et al. Seroprevalence of *Helicobacter pylori* in South Korea. Helicobacter. 2007;12:333–40.
38. Lim SH, Kwon JW, Kim N, Kim GH, Kang JM, Park MJ, et al. Prevalence and risk factors of *Helicobacter pylori* infection in Korea: nationwide multicenter study over 13 years. BMC Gastroenterol. 2013;13:104.
39. Kim JH, Kim HY, Kim NY, Kim SW, Kim JG, Kim JJ, et al. Seroepidemiological study of *Helicobacter pylori* infection in asymptomatic people in South Korea. J Gastroenterol Hepatol. 2001;16:969–75.

Part VIII

Consequences of *H. pylori* Eradication

Peptic Ulcer Disease

54

Jinjoo Kim

Abstract

Since the discovery of *Helicobacter pylori* (*H. pylori*), *H. pylori* eradication has dramatically lowered the recurrence rate of peptic ulcer disease (PUD). Combining *H. pylori* eradication with gastric acid inhibition accelerates ulcer healing compared to gastric acid inhibition alone. Moreover, *H. pylori* eradication prevents various PUD complications, such as bleeding, perforation, and obstruction. Therefore, for PUD patients with *H. pylori* infection, *H. pylori* eradication has become the standard care of treatment ensuring improved outcomes including ulcer healing and prevention of recurrence and complications.

Keywords

Helicobacter pylori · Peptic ulcer disease · Gastric acid

54.1 Introduction

Peptic ulcer disease (PUD) has been one of the most important causes of non-variceal upper gastrointestinal bleeding. *Helicobacter pylori* (*H. pylori*) infection is the major cause of PUD, along with the medications including nonsteroidal anti-inflammatory drugs (NSAIDs) and antithrombotic drugs [1]. Patients infected with *H. pylori* who also take such medications face an elevated risk of bleeding [2]. This chapter will explore the relationship between *H. pylori* and PUD pathogenesis, as well as the impact of its eradication on PUD healing/recurrence/complications.

54.2 The Role of *H. pylori* infection in Peptic Ulcer Disease Pathogenesis

Even though it varies according to the characteristics of the population, PUD usually occurs less than 1% per year, owing to the balance between the acid-peptic environment and protective mechanisms. Whether this mechanism is out of balance is the main issue in the pathogenesis of PUD. Disruption of this equilibrium lies at the heart of PUD pathogenesis, often triggered by factors such as *H. pylori* infection and the use of NSAIDs. While majority of *H. pylori* infected patients develop pangastritis, approximately 10–15% present with antrum predominant gastritis. This alteration leads to decreased antral somatostatin concentrations, heightened basal/stimulated gastric acid secretion, and ultimately contributes to the development of PUD [3–5].

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54.3 Impact on Peptic Ulcer Disease Healing

In a cohort study by Gisbert et al. [6] involving 212 duodenal ulcer patients, 22 patients (10.4%) have recurred after 1 month following *H. pylori* eradication and only three patients have recurred after two months, reporting 98.1% cure rate. Similar findings across multiple studies suggest that duodenal ulcers heal more effectively with *H. pylori* eradication compared to acid inhibition therapy alone [7–10]. Furthermore, even cases of refractory duodenal ulcers resistant to gastric acid inhibition therapy have shown successful healing with *H. pylori* eradication altogether, persisting for up to four years [11–22].

While fewer studies have examined the association between gastric ulcer and *H. pylori* eradication, research by Labentz and Börsch [23] revealed a cure rate of 84.9% among *H. pylori*-eradicated gastric ulcer patients, contrasted with 60% among those not treated for *H. pylori*. Additional studies support the efficacy of *H. pylori* eradication in healing gastric ulcers, even without ongoing gastric acid inhibition therapy [24–35]. Furthermore, evidence suggests that persistent *H. pylori* infection significantly impedes gastric ulcer healing, particularly in cases involving NSAID usage [36–38].

54.4 Impact on Peptic Ulcer Disease Recurrence

Prior to the discovery of *H. pylori*, PUD has been notorious for its high recurrence rate [39–43]. Previous studies have highlighted a significant correlation between successful *H. pylori* eradication and reduced recurrence of PUD [44, 45].

In a meta-analysis conducted by Hopkins et al. [46], the recurrence rate for duodenal ulcers was reported at 67% without *H. pylori* eradication, decreasing sharply to 6% following successful eradication. Similarly, for gastric ulcer, the recurrence rate dropped from 59% to 6% with successful eradication, as demonstrated by Hopkins [5, 46]. These findings underscore the pivotal role of *H. pylori* eradication as the cornerstone of standard PUD treatment to mitigate recurrence rates for both duodenal and gastric ulcers.

54.5 Impact on Peptic Ulcer Disease Complications

PUD is associated with several significant complications, including bleeding in 15–20%, perforation in 5%, and obstruction in 2% [47].

54.5.1 Bleeding

Studies by Murray et al. [48] and Jensen et al. [49] have demonstrated a high rebleeding rate among patients prescribed persistent ranitidine 150 mg. In comparison, research comparing patients receiving gastric acid inhibition alone versus those with successful *H. pylori* eradication found rebleeding rates of 12% and 2.3%, respectively. These findings suggest that *H. pylori* eradication not only reduce PUD recurrence but also lowers the incidence of bleeding-related complications [50–53].

54.5.2 Perforation

Although perforation is a severe complication, its incidence is relatively low, resulting in limited research on its relationship with *H. pylori* eradication. According to the study of patients with PUD perforation, those treated with cimetidine for gastric acid suppression showed significantly better prognosis than those without gastric acid inhibition, but this study has not considered *H. pylori* infection as a factor [54]. Studies have shown that NSAID poses a higher risk for PUD perforation compared to *H. pylori* infection [55–57]. According to previous studies with PUD perforation patients, the infection rate of *H. pylori* is quite high ranged from 50 to 80% [58–62]. Despite high rates of *H. pylori* infection among PUD perforation patients, research from Hong

Kong indicates a significantly lower recurrence rate (5%) in patients treated with both gastric acid inhibition and *H. pylori* eradication, compared to those treated with gastric acid inhibition alone (38%) [63].

54.5.3 Obstruction

Obstruction due to PUD occurs not that often either, which is less than 5%. Thus, studies on the relationship between PUD obstruction and *H. pylori* infection are quite limited so far [64–67]. PUD patients with obstruction showed from 45 to 90% *H. pylori* infection rate, and research indicates that successful relief of obstruction in PUD patients can be achieved through *H. pylori* eradication, with or without balloon dilatation [67–69].

54.6 Conclusions

In conclusion, the discussion has focused on the relationship between *H. pylori* eradication and PUD. It has been demonstrated that *H. pylori* eradication not only decreases the recurrence of PUD but also mitigates associated complications. Consequently, *H. pylori* eradication stands as the standard therapy for PUD patients with *H. pylori* infection.

References

1. Lanas A, Dumonceau JM, Hunt RH, Fujishiro M, Scheiman JM, Gralnek IM, et al. Non-variceal upper gastrointestinal bleeding. Nat Rev Dis Primers. 2018;4:18020.
2. Venerito M, Schneider C, Costanzo R, Breja R, Rohl F-W, Malfertheiner P. Contribution of *Helicobacter pylori* infection to the risk of peptic ulcer bleeding in patients on nonsteroidal anti-inflammatory drugs, antiplatelet agents, anticoagulants, corticosteroids and selective serotonin reuptake inhibitors. Aliment Pharmacol Ther. 2018;47:1464–71.
3. El-Omar EM, Panman ID, Ardill JE, Chittajallu RS, Howie C, McColl KE. *Helicobacter pylori* infection and abnormalities of acid secretion in patients with duodenal ulcer disease. Gastroenterology. 1995;109:681–91.
4. Moss SF, Legon S, Bishop AE, Polak JM, Calam J. Effect of *Helicobacter pylori* on gastric somatostatin in duodenal ulcer disease. Lancet. 1992;340:930–2.
5. Gillen D, El-Omar EM, Wirz AA, Ardill JE, McColl KE. The acid response to gastrin distinguish duodenal ulcer patients from *Helicobacter pylori*-infected healthy subjects. Gastroenterology. 1998;114:50–7.
6. Gisbert JP, Boixeda D, Martín De Argila C, Alvarez Baleriola I, Abraira V, García PA. Unhealed duodenal ulcers despite *Helicobacter pylori* eradication. Scand J Gastroenterol. 1997;32:643–50.
7. Graham DY, Lew GM, Evans DG, Evans DJ Jr, Klein PD. Effect of triple therapy (antibiotics plus bismuth) on duodenal ulcer healing. A randomized controlled trial. Ann Intern Med. 1991;115:266.
8. Marshall BJ, Goodwin CS, Warren JR, Murray R, Blincow ED, Blackbourn SJ, et al. Prospective double-blind trial of duodenal ulcer relapse after eradication of *Campylobacter pylori*. Lancet. 1988;2:1437–42.
9. Hentschel E, Brandstätter G, Dragosics B, Hirschl AM, Nemec H, Schütze K, et al. Effect of ranitidine and amoxicillin plus metronidazole on the eradication of *Helicobacter pylori* and the recurrence of duodenal ulcer. N Engl J Med. 1993;328:308–12.
10. Hosking SW, Ling TK, Yung MY, Cheng A, Chung SC, Leung JW, et al. Randomised controlled trial of short term treatment to eradicate *Helicobacter pylori* in patients with duodenal ulcer. BMJ. 1992;305:502–4.
11. Rauws EA, Tytgat GN. Cure of duodenal ulcer associated with eradication of *Helicobacter pylori*. Lancet. 1990;335:1233–5.
12. George LL, Borody TJ, Andrews P, Devine M, Moore-Jones D, Walton M, et al. Cure of duodenal ulcer after eradication of *Helicobacter pylori*. Med J Aust. 1990;153:145–9.
13. Bianchi Porro G, Parente F, Lazzaroni M. Short and long term outcome of *Helicobacter pylori* positive resistant duodenal ulcers treated with colloidal bismuth subcitrate plus antibiotics or sucralfate alone. Gut. 1993;34:466–9.
14. Mantzaris GJ, Hatzis A, Tamvakologos G, Petraki K, Spiliades C, Triadaphyllou G. Prospective, randomized, investigator-blind trial of *Helicobacter pylori* infection treatment in patients with refractory duodenal ulcers. Healing and long-term relapse rates. Dig Dis Sci. 1993;38:1132–6.
15. Wagner S, Gebel M, Haruma K, Bär W, Lange P, Freise J, et al. Bismuth subsalicylate in the treatment of H2 blocker resistant duodenal ulcers: role of *Helicobacter pylori*. Gut. 1992;33:179–83.
16. Labenz J, Börsch G. Highly significant change of the clinical course of relapsing and complicated peptic ulcer disease after cure of *Helicobacter pylori* infection. Am J Gastroenterol. 1994;89:1785–8.
17. Arkkila PE, Seppälä K, Kosunen TU, Sipponen P, Mäkinen J, Rautelin H, et al. *Helicobacter pylori* erad-

ication as the sole treatment for gastric and duodenal ulcers. Eur J Gastroenterol Hepatol. 2005;17:93–101.

18. Lam SK, Ching CK, Lai KC, Wong BC, Lai CL, Chan CK, et al. Does treatment of *Helicobacter pylori* with antibiotics alone heal duodenal ulcer? A randomised double blind placebo controlled study. Gut. 1997;41:43–8.
19. Hosking SW, Ling TK, Chung SC, Yung MY, Cheng AF, Sung JJ, et al. Duodenal ulcer healing by eradication of *Helicobacter pylori* without anti-acid treatment: randomised controlled trial. Lancet. 1994;343:508–10.
20. Logan RP, Gummett PA, Misiewicz JJ, Karim QN, Walker MM, Baron JH. One week's anti-*Helicobacter pylori* treatment for duodenal ulcer. Gut. 1994;35:15–8.
21. Laine L, Suchower L, Frantz J, Connors A, Neil G. Twice-daily, 10-day triple therapy with omeprazole, amoxicillin, and clarithromycin for *Helicobacter pylo*ri eradication in duodenal ulcer disease: results of three multicenter, double-blind. US Trials Am J Gastroenterol. 1998;93:2106–12.
22. Wheeldon TU, Hoang TT, Phung DC, Björkman A, Granström M, Sörberg M. *Helicobacter pylori* eradication and peptic ulcer healing: the impact of deleting the proton pump inhibitor and using a once-daily treatment. Aliment Pharmacol Ther. 2003;18:93–100.
23. Labenz J, Börsch G. Evidence for the essential role of *Helicobacter pylori* in gastric ulcer disease. Gut. 1994;35:19–22.
24. Malfertheiner P, Kirchner T, Kist M, Leodolter A, Peitz U, Strobel S, et al. *Helicobacter pylori* eradication and gastric ulcer healing—comparison of three pantoprazole-based triple therapies. Aliment Pharmacol Ther. 2003;17:1125–35.
25. Borody TJ, George LL, Brandl S, Andrews P, Ostapowicz N, Hyland L, et al. *Helicobacter pylori*-negative duodenal ulcer. Am J Gastroenterol. 1991;86:1154–7.
26. Penston JG, McColl KE. Eradication of *Helicobacter pylori*: an objective assessment of current therapies. Br J Clin Pharmacol. 1997;43:223–43.
27. Tatsuta M, Ishikawa H, Iishi H, Okuda S, Yokota Y. Reduction of gastric ulcer recurrence after suppression of *Helicobacter pylori* by cefixime. Gut. 1990;31:973–6.
28. Asaka M, Ohtaki T, Kato M, Kudo M, Kimura T, Meguro T, et al. Causal role of *Helicobacter pylori* in peptic ulcer relapse. J Gastroenterol. 1994;29 Suppl 7:134–8.
29. Sung JJ, Chung SC, Ling TK, Yung MY, Leung VK, Ng EK, et al. Antibacterial treatment of gastric ulcers associated with *Helicobacter pylori*. N Engl J Med. 1995;332:139–42.
30. Bayerdörffer E, Miehlke S, Lehn N, Mannes GA, Höchter W, Weingart J, et al. Cure of gastric ulcer disease after cure of *Helicobacter pylori* infection—German gastric ulcer study. Eur J Gastroenterol Hepatol. 1996;8:343–9.
31. Malfertheiner P, Bayerdörffer E, Diete U, Gil J, Lind T, Misiuna P, et al. The GU-MACH study: the effect of 1-week omeprazole triple therapy on *Helicobacter pylori* infection in patients with gastric ulcer. Aliment Pharmacol Ther. 1999;13:703–12.
32. Higuchi K, Fujiwara Y, Tominaga K, Watanabe T, Shiba M, Nakamura S, et al. Is eradication sufficient to heal gastric ulcers in patients infected with *Helicobacter pylori*? A randomized, controlled, prospective study. Aliment Pharmacol Ther. 2003;17:111–7.
33. Hsu CC, Lu SN, Changchien CS. One-week low-dose triple therapy without anti-acid treatment has sufficient efficacy on *Helicobacter pylori* eradication and ulcer healing. Hepato-Gastroenterology. 2003;50:1731–4.
34. Gisbert JP, Pajares JM. Systematic review and meta-analysis: is 1-week proton pump inhibitor-based triple therapy sufficient to heal peptic ulcer? Aliment Pharmacol Ther. 2005;21:795–804.
35. Tulassay Z, Stolte M, Sjölund M, Engstrand L, Butruk E, Malfertheiner P, et al. Effect of esomeprazole triple therapy on eradication rates of *Helicobacter pylori*, gastric ulcer healing and prevention of relapse in gastric ulcer patients. Eur J Gastroenterol Hepatol. 2008;20:526–36.
36. Tytgat GN. Review article: treatments that impact favourably upon the eradication of *Helicobacter pylori* and ulcer recurrence. Aliment Pharmacol Ther. 1994;8:359–68.
37. Leodolter A, Kulig M, Brasch H, Meyer-Sabellek W, Willich SN, Malfertheiner P. A meta-analysis comparing eradication, healing and relapse rates in patients with *Helicobacter pylori*-associated gastric or duodenal ulcer. Aliment Pharmacol Ther. 2001;15:1949–58.
38. Befrits R, Sjöstedt S, Tour R, Leijonmarck CE, Hedenborg L, Backman M, Stockholm United Study Group for Helicobacter pylori. Long-term effects of eradication of *Helicobacter pylori* on relapse and histology in gastric ulcer patients: a two-year follow-up study. Scand J Gastroenterol. 2004;39:1066–72.
39. Londong W, Barth H, Dammann HG, Hengels KJ, Kleinert R, Müller P, et al. Dose-related healing of duodenal ulcer with the proton pump inhibitor lansoprazole. Aliment Pharmacol Ther. 1991;5:245–54.
40. Bardhan KD, Bianchi Porro G, Bose K, Daly M, Hinchliffe RF, Jonsson E, et al. A comparison of two different doses of omeprazole versus ranitidine in treatment of duodenal ulcers. J Clin Gastroenterol. 1986;8:408–13.
41. Misra SC, Dasarathy S, Sharma MP. Omeprazole versus famotidine in the healing and relapse of duodenal ulcer. Aliment Pharmacol Ther. 1993;7:443–9.
42. Dammann HG, Walter TA. Efficacy of continuous therapy for peptic ulcer in controlled clinical trials. Aliment Pharmacol Ther. 1993;7 Suppl 2:17–25.
43. Penston JG, Wormsley KG. Nine years of maintenance treatment with ranitidine for patients with duodenal ulcer disease. Aliment Pharmacol Ther. 1992;6:629–45.

44. Zanten SJ, Bradette M, Farley A, Leddin D, Lind T, Unge P, et al. The DU-MACH study: eradication of *Helicobacter pylori* and ulcer healing in patients with acute duodenal ulcer using omeprazole based triple therapy. Aliment Pharmacol Ther. 1999;13:289–95.
45. Coghlan JG, Gilligan D, Humphries H, McKenna D, Dooley C, Sweeney E, et al. *Campylobacter pylori* and recurrence of duodenal ulcers-a 12- month follow-up study. Lancet. 1987;2:1109–11.
46. Hopkins RJ, Girardi LS, Turney EA. Relationship between *Helicobacter pylori* eradication and reduced duodenal and gastric ulcer recurrence: a review. Gastroenterology. 1996;110:1244–52.
47. Laine LA. *Helicobacter pylori* and complicated ulcer disease. Am J Med. 1996;100:52–9.
48. Murray WR, Cooper G, Laferla G, Rogers P, Archibald M. Maintenance ranitidine treatment after haemorrhage from a duodenal ulcer. A 3-year study. Scand J Gastroenterol. 1988;23:183–7.
49. Jensen DM, Cheng S, Kovacs TO, Randall G, Jensen ME, Reedy T, et al. A controlled study of ranitidine for the prevention of recurrent hemorrhage from duodenal ulcer. N Engl J Med. 1994;330:382–6.
50. Kikkawa A, Iwakiri R, Ootani H, Ootani A, Fujise T, Sakata Y, et al. Prevention of the rehaemorrhage of bleeding peptic ulcers: effects of *Helicobacter pylori* eradication and acid suppression. Aliment Pharmacol Ther. 2005;21 Suppl 2:79–84.
51. Gisbert JP, Khorrami S, Carballo F, Calvet X, Gené E, Dominguez-Muñoz JE. *H. pylori* eradication therapy vs. antisecretory non-eradication therapy (with or without long-term maintenance antisecretory therapy) for the prevention of recurrent bleeding from peptic ulcer. Cochrane Database Syst Rev. 2004;2:CD004062.
52. Horvat D, Vcev A, Soldo I, Timarac J, Dmitrović B, Misević T, et al. The results of *Helicobacter pylori* eradication on repeated bleeding in patients with stomach ulcer. Coll Antropol. 2005;29:139–42.
53. Gisbert JP, Calvet X, Feu F, Bory F, Cosme A, Almela P, et al. Eradication of *Helicobacter pylori* for the prevention of peptic ulcer rebleeding. Helicobacter. 2007;12:279–86.
54. Simpson CJ, Lamont G, Macdonald I, Smith IS. Effect of cimetidine on prognosis after simple closure of perforated duodenal ulcer. Br J Surg. 1987;74:104–5.
55. Svanes C, Ovrebø K, Søreide O. Ulcer bleeding and perforation: non-steroidal anti-inflammatory drugs or *Helicobacter pylori*. Scand J Gastroenterol. 1996;220:128–31.
56. Kumar S, Mittal GS, Gupta S, Kaur I, Aggarwal S. Prevalence of *Helicobacter pylori* in patients with perforated duodenal ulcer. Trop Gastroenterol. 2004;25:121–4.
57. Reinbach DH, Cruickshank G, McColl KE. Acute perforated duodenal ulcer is not associated with *Helicobacter pylori* infection. Gut. 1993;34:1344–7.
58. Kate V, Ananthakrishnan N, Badrinath S. Effect of *Helicobacter pylori* eradication on the ulcer recurrence rate after simple closure of perforated duodenal ulcer: retrospective and prospective randomized controlled studies. Br J Surg. 2001;88:1054–8.
59. Metzger J, Styger S, Sieber C, von Flüe M, Vogelbach P, Harder F. Prevalence of *Helicobacter pylori* infection in peptic ulcer perforations. Swiss Med Wkly. 2001;131:99–103.
60. Sebastian M, Chandran VP, Elashaal YI, Sim AJ. *Helicobacter pylori* infection in perforated peptic ulcer disease. Br J Surg. 1995;82:360–2.
61. Ng EK, Chung SC, Sung JJ, Lam YH, Lee DW, Lau JY, et al. High prevalence of *Helicobacter pylori* infection in duodenal ulcer perforations not caused by non-steroidal anti-inflammatory drugs. Br J Surg. 1996;83:1779–81.
62. Matsukura N, Onda M, Tokunaga A, Kato S, Yoshiyuki T, Hasegawa H, et al. Role of *Helicobacter pylori* infection in perforation of peptic ulcer: an age- and gender-matched case-control study. J Clin Gastroenterol. 1997;25:S235–9.
63. Ng EK, Lam YH, Sung JJ, Yung MY, To KF, Chan AC, et al. Eradication of *Helicobacter pylori* prevents recurrence of ulcer after simple closure of duodenal ulcer perforation: randomized controlled trial. Ann Surg. 2000;231:153–8.
64. de Boer WA, Driessen WM. Resolution of gastric outlet obstruction after eradication of *Helicobacter pylori*. J Clin Gastroenterol. 1995;21:329–30.
65. Annibale B, Marignani M, Luzzi I, Delle Fave GF. Peptic ulcer and duodenal stenosis: role of *Helicobacter pylori* infection. Ital J Gastroenterol. 1995;27:26–8.
66. Lam Y, Lau JY, Law KB, Sung JJ, Chung SS. Endoscopic balloon dilation and *Helicobacter pylori* eradication in the treatment of gastric outlet obstruction. Gastrointest Endosc. 1997;46:379–80.
67. Taskin V, Gurer I, Ozyilkan E, Sare M, Hilmioglu F. Effect of *Helicobacter pylori* eradication on peptic ulcer disease complicated with outlet obstruction. Helicobacter. 2000;5:38–40.
68. Gisbert JP, Pajares JM. *Helicobacter pylori* infection and gastric outlet obstruction – prevalence of the infection and role of antimicrobial treatment. Aliment Pharmacol Ther. 2002;16:1203–8.
69. Cherian PT, Cherian S, Singh P. Long-term follow-up of patients with gastric outlet obstruction related to peptic ulcer disease treated with endoscopic balloon dilatation and drug therapy. Gastrointest Endosc. 2007;66:491–7.

Atrophic Gastritis and Intestinal Metaplasia

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Nayoung Kim and Hyuk Yoon

Abstract

Currently, many international guidelines recommend *Helicobacter pylori* (*H. pylori*) eradication in patients with precancerous lesions such as atrophic gastritis (AG) and intestinal metaplasia (IM). However, there are still many controversies in whether *H. pylori* eradication improves AG and IM. Taking the results of related studies together, it seems likely that *H. pylori* eradication improves AG. However, a dominant opinion has been that IM which progressed to a certain level is already an irreversible status (point of no return). Recently, there were major reports that IM also seemed to improve when the follow-up period became longer than at least 5 years after *H. pylori* eradication. The main mechanism of reversibility of IM may be associated with a decrease in the CDX2 mRNA expression. The decrease of AG and IM by *H. pylori* eradication could be affected by other factors such as young age, high income, and *cagA*-positive status. Further research regarding the reversibility of IM and its underlying mechanism is needed.

Keywords

Atrophic gastritis · Eradication · *Helicobacter pylori* · Intestinal metaplasia · CDX1 · CDX2

55.1 Introduction

Helicobacter pylori (*H. pylori*) is well known as a major cause of gastric cancer (GC). Therefore, whether the eradication of *H. pylori* could reverse atrophic gastritis (AG) and intestinal metaplasia (IM) which are precancerous lesions and could inhibit the progression of these lesions are clinically important topics. However, conflicting results have been presented concerning whether AG and IM are improved by *H. pylori* eradication [1–5]. In some reports, *H. pylori* eradication improved AG and IM [4, 6–10] but not in other studies [5, 11–14]. Thus, Wong et al. have suggested a "point of no return" in terms of the effect of the *H. pylori* eradication for GC prevention [2]. Kiriyama et al. have also stated that mucosal damage of the IM stage cannot improve to normal gastric mucosa due to "histological point of no return." [11]. However, these studies had limitations in study design, including small cohorts or insufficient follow-up periods with histological diagnosis [12, 15]. Recently, the long-term follow-up studies clearly showed that *H. pylori* eradication improved AG [16, 17]. Furthermore, IM also seemed to improve when the follow-up period became longer than at least 5 years after

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the *H. pylori* eradication [16, 17]. This decrease of AG and IM by the *H. pylori* eradication looks like to be affected by other factors such as female sex, young age, high income, and non-smoking [14, 18–20]. CDX1 and CDX2 may play a crucial role in the development, maintenance, proliferation, and anterior–posterior patterning of the intestinal epithelium [21]. While CDX2 is highly expressed in the small intestine and its expression declines when progressing distally in the colon, the opposite is observed for CDX1 [21]. An aberrant expression of CDX1 and CDX2 in gastric mucosae is related to *H. pylori* infection and IM, which plays a role in gastric carcinogenesis [22]. Thus, the main mechanism of reversibility of IM was suggested to be a decrease in the CDX2 mRNA expression [21]. In this chapter, the effect of the *H. pylori* eradication on the AG and IM will be updated in terms of key individual studies, meta-analyses, and major guidelines all over the world. In addition, the main mechanism of reversibility of AG and IM will be introduced in terms of CDX1 and CDX2.

55.2 The Effect of *H. pylori* Eradication on the Atrophic Gastritis and Intestinal Metaplasia

Several reports have been continuously coming out regarding the effect of *H. pylori* eradication on the AG and IM in the forms of key individual studies and meta-analyses. After some time, they are reflected in the guidelines in the world.

55.2.1 Key Individual Studies

H. pylori eradication is the basis of treatment of AG and IM. However, it is still a controversial topic with a question of whether *H. pylori* eradication can improve AG and IM [4, 7, 13, 16, 17, 23–35] (Table 55.1). Several clinical studies reported that the eradication of *H. pylori* can improve AG and IM which are the main precancerous lesions [36, 37]. The cohort study by Correa et al. is the first well-designed study in this field. The authors treated *H. pylori* infection in 795 adults who lived in an area with high risk for GC in Colombia and had precancerous lesions and followed them up for 12 years [36]. The rate of regression or delayed progression of AG and IM was significantly higher in patients who showed successful eradication of *H. pylori* than in patients who failed to eradicate *H. pylori* [36, 37]. A randomized clinical study for 3365 people in China also suggested that the *H. pylori* eradication significantly reduced the prevalence of AG and IM after an 8-year follow-up [38]. Another Chinese study reported that the *H. pylori* eradication is effective in delaying the progression of IM after a 5-year follow-up [5]. In contrast, a recent Taiwanese study suggested that the *H. pylori* eradication in mass population did not decrease IM after a 4-year follow-up, even though it significantly reduced AG [39]. However, when they prolonged the follow-up period up to 2018, the results became different [17]. That is, when they analysed AG and IM according to the operative link for the gastritis assessment of atrophic gastritis (OLGA) and operative link for the gastritis assessment of intestinal metaplasia (OLGIM) grading systems over 2004–2018, the prevalence rates of AG and IM declined from 55.9% to 15.9% and from 31.7% to 21.4%, respectively (both $p < 0.001$).

In addition, a famous randomized clinical study by Wong et al. showed that the *H. pylori* eradication decreased the incidence of GC only in subjects without initial precancerous lesions such as AG and IM [2]. In this report, the authors treated *H. pylori*-infected subjects among the general population in 2004 and followed them up for 4 years [2]. However, when they prolonged the follow-up period up to 2018, the results also became different. That is, the presence and severity of AG and IM also decreased with time [40]. Compared with the historical control period from 1995 to 2003, the effectiveness in reducing GC incidence and mortality during the chemoprevention period was 53% (95% confidence interval [CI], 30–69%; $p < 0.001$) and (95% CI, 14–51%; $p = 0.18$), respectively [40]. Our team also found similar results. That is, when the follow-up period was short like within 3 years, only AG improved

Table 55.1 Histologic parameters of AG and IM after *Helicobacter pylori* eradication

	Study arms (*n*)			Histologic parameters			
				AG		IM	
Author, year (country)	Eradicated	Not eradicated	Follow-up (months)	Antrum	Corpus	Antrum	Corpus
Sung et al. 2000 (China) [4]	226	245	12	Yes	Yes	Yes	Yes
Kim et al. 2000 (South Korea) [23]	41	16	24	No	No	Yes	Yes
Annibale et al. 2000 (Italy) [24]	25	7	6	Yes	Yes	Yes	Yes
Ohkusa et al. 2001 (Japan) [25]	115	48	12–15	Yes	Yes	Yes	Yes
Ruiz et al. 2001 (Colombia) [26]	29	21	72	Yes	No	No	No
Ito et al. 2002 (Japan) [27]	22	22	60	Yes	Yes	Yes	Yes
Annibale et al. 2002 (Italy) [28]	40	0	6–12	Yes	Yes	Yes	Yes
Yamada et al. 2003 (Japan) [29]	87	29	10–50	Yes	Yes	Yes	Yes
Iacopini et al. 2003 (Italy) [30]	10	0	12	Yes	No	Yes	No
Kamada et al. 2003 (Japan) [31]	37	8	36	Yes	No	No	No
Wambura et al. 2004 (Japan) [32]	107	118	12	Yes	Yes	Yes	Yes
Lahneret al. 2005 (Italy) [13]	38	36	48–137	Yes	Yes	Yes	Yes
Lu et al. 2005 (China) [7]	92	87	36	Yes	Yes	Yes	Yes
Kamada et al. 2005 (Japan) [33]	1787	233	12	Yes	Yes	Yes	Yes
Toyokawa et al. 2009 (Japan) [15]	241	19	60	Yes	Yes	Yes	Yes
Kang et al. 2012 (South Korea) [12]	210	74	36	Yes	Yes	Yes	Yes
Hwang et al. 2018 (South Korea) [16]	442	91	77.4	Yes	Yes	Yes	Yes

but IM did not [12]. However, when the follow-up period became longer up to 10 years, then IM also significantly improved [16]. In detail, histological follow-up was performed regularly at 1, 2, 3–4, and ≥ 5 years, AG in antrum and corpus gradually and significantly (both $p < 0.05$ for all time points) improved only in the *H. pylori*-eradicated group compared to that at baseline [16]. Significant difference in AG between *H. pylori*-eradicated and *H. pylori*-negative groups disappeared from 1-year follow-up [16] (Fig. 55.1). Similarly, IM in antrum and corpus also improved significantly (both $p < 0.05$ for all time points) only in the *H. pylori*-eradicated group in comparison with that at baseline [16] (Fig. 55.1). Significant difference in IM between *H. pylori*-eradicated and *H. pylori*-negative groups disappeared from ≥5 years of follow-up in the antrum and from 3 years of follow-up in the corpus [16] (Fig. 55.1).

In South Korea, there was a nice report that *H. pylori* eradication treatment reduced the risk of GC among persons with *H. pylori* infection who had a family history of GC in first-degree relatives [41]. In detail, a total of 1676 participants were included in the modified intention-to-treat population for the analysis of the primary outcome (832 in the treatment group and 844 in the placebo group) [41]. During a median follow-up of 9.2 years, GC developed in 10 participants (1.2%) in the treatment group and in 23 (2.7%) in the placebo group (HR 0.45; 95% CI, 0.21–0.94; $p = 0.03$ by log-rank test). Among the 10 participants in the treatment group in whom gastric cancer developed, five (50.0%) had persistent *H. pylori* infection. GC developed in 0.8% of participants (5 of 608) in whom *H. pylori* infection was eradicated and in 2.9% of participants (28 of 979) who had persistent infection (HR 0.27; 95% CI, 0.10–0.70) [41].

Taken together, these recent findings imply that *H. pylori* eradication could be effective for the prevention of GC in a high-risk group with precancerous lesions. In spite of these results, patients with AG and/or IM should keep receiving surveillance endoscopy because GC occur even after the *H. pylori* eradication [42]. A test-to-treat strategy to perform the *H. pylori* eradication in the young population before the development of AG and IM might be more effec-

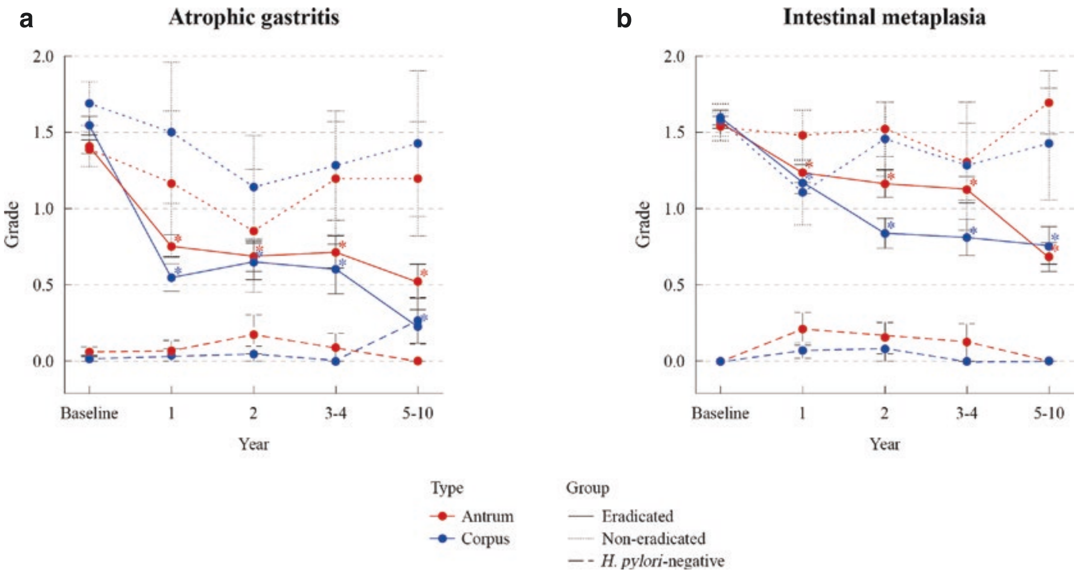

Fig. 55.1 Serial changes of grades for atrophic gastritis (**a**) and intestinal metaplasia (**b**) in the antrum and the corpus depending on *H. pylori* eradication in the 548 subjects without gastric cancer or dysplasia [*H. pylori*-negative ($n = 65$), non-*H. pylori*-eradicated ($n = 91$), and *H. pylori*-eradicated ($n = 442$)] with long-term follow-up for up to 10 years (Adapted from Hwang et al. [16])

tive for the prevention of GC [43]. However, an analysis of data from a public hospital database suggested a benefit of survival by *H. pylori* eradication therapy in older subjects (60 years or older) who are the highest risk group, 10 or more years after treatment [44]. These results also suggest that the *H. pylori* eradication therapy is effective for the primary prevention of GC even after the development of AG and/or IM.

Because a considerable fraction of GC patients already has an advanced stage of AG and IM, we can infer the effect of the *H. pylori* eradication on AG and IM by examining the incidence of metachronous GC in GC patients who received the *H. pylori* eradication. One retrospective Japanese study reported that the *H. pylori* eradication in GC patients who underwent the endoscopic resection of early gastric cancer (EGC) decreased the incidence of metachronous GC [45]. Thereafter, Fukase et al. published the article suggesting that compared with the control group, the odds ratio (OR) for the incidence of metachronous GC in the patients who received *H. pylori* eradication after endoscopic submucosal dissection of EGC was 0.35 (95% CI, 0.16–0.78; $p = 0.009$) [46]. This article has been an evidence for recommending the *H. pylori* eradication in EGC patients in Japan. However, another retrospective Japanese study concluded that when they followed-up the patients who underwent endoscopic submucosal dissection of EGC for a long time, *H. pylori* eradication did not reduce the incidence of metachronous GC [47]. The results of randomized controlled study which evaluated the effect of *H. pylori* eradication on the incidence of metachronous GC in 901 patients who underwent endoscopic resection of EGC was recently published in Korea [48]. During the median 3 years of follow-up period, there was no significant difference in the incidence of metachronous GC between the *H. pylori* eradication group and the control group [48]. However, another South Korean randomized controlled trial (RCT) reported that the *H. pylori* eradication reduced the incidence of metachronous GC in the patients who underwent the endoscopic resection of EGC [49]. In addition, in the RCT, patients with EGC who received *H. pylori* treatment showed lower rates of metachronous GC and more improvement from baseline in the grade of gastric corpus atrophy than patients who received placebo [50]. In detail, a total of 396 patients were included in the modified intention-to-treat analysis population (194 in the treatment group and 202 in the placebo group). During a median follow-up of 5.9 years, metachronous GC developed in 14 patients (7.2%) in the treatment group and in 27 patients (13.4%) in the placebo group (hazard ratio in the treatment group, 0.50; 95% CI, 0.26–0.94; $p = 0.03$). Among the 327 patients in the subgroup that underwent histologic analysis, improvement from baseline in the atrophy grade at the gastric corpus lesser curvature was observed in 48.4% of the patients in the treatment group and in 15.0% of those in the placebo group ($p < 0.001$) [50]. Recently, the results of meta-analysis in 13 studies including 6237 subjects which evaluated the effect of the *H. pylori* eradication on the development of metachronous GC in the subjects who underwent endoscopic resection of EGC were reported [51]. They reported that the *H. pylori* eradication in the patients who underwent endoscopic resection of EGC decreased the incidence of metachronous GC approximately by half (OR 0.42; 95% CI, 0.32–0.56) [51]. Maybe, there are several underlying mechanisms of the secondary chemoprevention effect of the *H. pylori* eradication. One of these mechanisms might be the reversibility of AG and IM after the *H. pylori* eradication [52].

55.2.2 Meta-Analyses

Until now, three meta-analyses evaluated the effect of the *H. pylori* eradication on AG and IM. The first meta-analysis by Rokkas et al. in 2007 included one randomized controlled trial and seven observational studies of a total of 1154 subjects [53]. All included studies evaluated the degree of AG and IM using the updated Sidney system. The improvement of AG in the *H. pylori* eradication group was significant compared to the control group; the OR of AG in the *H. pylori* eradication group was 0.554 (95% CI, 0.372–0.825; $p = 0.004$) in the antrum and 0.209 (95%

CI, 0.081–0.538; $p < 0.001$) in the corpus. However, the OR for IM was not significantly different between the *H. pylori* eradication group and the control group; the OR of IM in the *H. pylori* eradication group was 0.795 (95% CI, 0.587–1.078; $p = 0.14$) in the antrum and 0.891 (95% CI, 0.663–1.253; $p = 0.506$) in the corpus. Therefore, the authors concluded that the *H. pylori* eradication can improve AG but it cannot reverse IM. A second meta-analysis by Wang et al. in 2011 evaluated three randomized controlled studies and nine observational studies of a total of 2658 subjects [54]. When the histological changes of gastric mucosa were compared before and after the *H. pylori* eradication, the pooled weighted mean difference of AG was 0.12 (95% CI, 0.00–0.23; $p = 0.06$) in the antrum and 0.32 (95% CI, 0.09–0.54; $p = 0.006$) in the corpus, respectively. The pooled weighted mean difference of IM was 0.02 (95% CI, −0.12–0.16; $p = 0.76$) in the antrum and − 0.02 (95% CI, −0.05–0.02; $p = 0.42$) in the corpus, respectively. That is, the *H. pylori* eradication could improve only AG in the corpus among precancerous lesions. Considering that AG generally starts in the antrum and progresses to the corpus, these results could be interpreted that the recent onset atrophy could be reversible first. The remote onset atrophy in the antrum takes long time for the recovery. In contrast to these two meta-analyses which suggested IM as a "point of no return," a more recent meta-analysis by Kong et al. suggested that there were improvements in IM after the *H. pylori* eradication [55].. In this meta-analysis, a total of 4294 subjects and 16 studies were included. The pooled weighted mean difference of AG in the *H. pylori* eradication group was 0.25 (95% CI, 0.15–0.35; $p < 0.0001$) in the antrum and 0.14 (95% CI, 0.04–0.24; $p = 0.008$) in the corpus; the improvement of AG was significant compared with the control group [55]. IM in the antrum was also improved (the pooled weighted mean difference 0.23 (95% CI, 0.18–0.29; $p < 0.0001$). However, IM in the corpus was not different (the pooled weighted mean difference − 0.01 (95% CI, −0.04–0.02; $p = 0.51$)). This meta-analysis has strong points in that it included more subjects than previous meta-analyses and it applied a stricter histologic method in evaluating AG and IM; it included studies which collected more than three pieces of gastric mucosal samples in each area [55].

Taking these three meta-analyses together, it seems likely that the *H. pylori* eradication can improve AG to some extent, but it cannot improve IM, especially advanced IM in the corpus. However, there are many limitations in these meta-analyses. First, the individual studies included were mostly from Japan and Italy and performed in a single institution. Second, although the histological evaluation of antral AG was performed in all studies, many studies did not evaluate corpus AG and IM. Third, the meta-analysis by Kong et al. included studies showed heterogeneity for AG [54]. Fourth, although gastric carcinogenesis occurs over a long period of time, the follow-up period in most studies was very short, approximately just 1 year. Finally, most studies were observational ones and randomized controlled trials were rare. Recent studies regarding this issue showed that if the follow-up was longer at least 5 years, then the reversibility of IM as well as AG became significant [16, 17, 40]. New meta-analysis will be coming including these recent positive results. Therefore, making a definite conclusion is rather difficult in the present situation.

55.2.3 Guidelines

The guideline which was presented in 2012 by ESGE (European Society of Gastrointestinal Endoscopy), EHSG (European Helicobacter Study Group), and ESP (European Society of Pathology) suggested that the *H. pylori* eradication can partially regress AG but it cannot reverse IM [56]. However, at the same time, this guideline recommended the *H. pylori* eradication in patients with IM based on the reason that the *H. pylori* eradication might delay progression to neoplasm in these patients. For the management of *H. pylori* infection, the Maastricht IV/Florence Consensus Report which was published in a similar time concluded that there are some controversies in whether the *H. pylori* eradication could reverse AG and there was no evidence supporting the *H. pylori* eradication could reverse IM [8]. Nevertheless, this report also included AG and IM in the indication of the *H. pylori* eradication. The Maastricht VI/Florence Consensus Report in 2022 showed more aggressive approach for the prevention of progression of AG and IM by the *H. pylori* eradication [57]. With regard to the Asian view, guidelines of China, Japan, and Korea in 2013 all recommended the *H. pylori* eradication in patients with AG and IM [43, 58, 59]. In addition, the revied Korean guideline 2020 also categorized AG and IM as the admissive indication of the *H. pylori* eradication [60].

55.2.4 Limitations

We can think of several reasons regarding why the effect of the *H. pylori* eradication on AG and IM varies with studies. First, interobserver variations in the endoscopic diagnosis of AG and IM are very high. In addition, in many cases, these precancerous lesions show different distributions according to each area of the stomach [61, 62]. Therefore, to evaluate the change of these precancerous lesions precisely before and after *H. pylori* eradication is very difficult. Second, it is known that the possibility to show improvement in AG and IM is higher when the *H. pylori* eradication was performed in the early stage than in the advanced stage [63]. As an example supporting this notion, in a Japanese study which performed the *H. pylori* eradication in 1674 patients with peptic ulcer and followed them up for a mean 5.6 years, the risk ratio of GC increased in proportion to the degree of atrophy of baseline gastric mucosa [64]. Third, among the three types of IM, type III (incomplete colonic type) is known to have a high risk for progression to GC. A study which analysed the results according to the types of IM showed that the complete type of IM is more reversible after *H. pylori* eradication than the incomplete type of IM [65]. However, most studies which evaluated the effect of *H. pylori* eradication on the precancerous lesions did not classify subtypes of AG and IM. Nevertheless, some authors suggest that the *H. pylori* eradication is meaningful in the precancerous lesions since the *H. pylori* eradication makes the gastric mucosa clearer by reversing some portions of the precancerous lesions and therefore relatively small cancer could be more easily detected [66].

55.3 Affecting Factors on the Decrease of Atrophic Gastritis and Intestinal Metaplasia by *H. pylori* Eradication

Experts think of reasons for these various results of *H. pylori* eradication effect on the AG and IM. That is, there might be affecting factors on these. One report analysed the affecting on the decrease of AG and IM in 435 subjects (220 in the PPI triple therapy and 215 in the placebo group) available for analysis, 10 developed GC and three had dysplasia [5]. Overall progression of IM was noted in 52.9% of subjects [5]. With multiple logistic regression, duodenal ulcer (OR 0.23; 95% CI, 0.09–0.58) was found to be an independent protective factor against IM progression [5]. Conversely, persistent *H. pylori* infection (OR 2.13; 95% CI, 1.41–3.24), age > 45 years (OR 1.92; 95% CI, 1.18–3.11), alcohol use (OR 1.67; 95% CI, 1.07–2.62), and drinking water from a well (OR 1.74; 95% CI, 1.13–2.67) were independent risk factors associated with IM progression [5]. When our team analysed these factors the decrease of AG and IM by *H. pylori* eradication could be affected by other factors such as young age, high income, and *cagA*-positive status [14, 18–20]. In addition, AG/IM of female sex decreased more than that of male sex because of they showed weaker AG and IM grade and eat frequently vegetables and fruits and less smoking and alcohol drinking [20].

Another point is the diagnostic methods of AG and IM. Most experts value the histology than endoscopic improvement. When our team performed the comparison of endoscopic finding and histologic findings after *H. pylori* eradication, the effect was different between these two methods [67]. That is, in a total of 380 subjects without significant gastrointestinal diseases were prospectively enrolled for up to 12 years and grouped by their *H. pylori* infection status: negative, non-eradicated, and eradicated [67]. First of all, endoscopic and histologic analyses of AG and IM were performed in the antrum and the corpus, by annual follow-up endoscopy first of all endoscopic AG and IM in the antrum and corpus in the eradicated group improved, compared to that in the non-eradicated group (AG, $p = 0.002$ and $p = 0.005$; IM, $p = 0.038$ and $p = 0.048$, respectively). Histologic AG and IM in the antrum and corpus in the eradicated group also improved, compared to that in the non-eradicated group (all $p < 0.001$) [67] (Fig. 55.2). Time taken to the endoscopic improvement of AG and IM after the *H. pylori* eradication was significantly longer than time taken to the histologic improvement in the antrum and corpus (AG in antrum: 3.47 ± 2.60 vs. 2.34 ± 1.71 years; $p = 0.004$; AG in corpus: 3.19 ± 2.30 vs. 1.87 ± 1.48 years; $p = 0.002$; IM in antrum: 4.40 ± 2.38 vs. 3.62 ± 2.35 years; $p = 0.043$; and IM in corpus: 4.82 ± 1.08 vs. 3.61 ± 2.22 years; $p = 0.007$, respectively) [67]. These results suggest that both endoscopic and histologic improvements of AG and IM were observed after the *H. pylori* eradication, while endoscopic improvement took significantly longer time than histologic improvement [67].

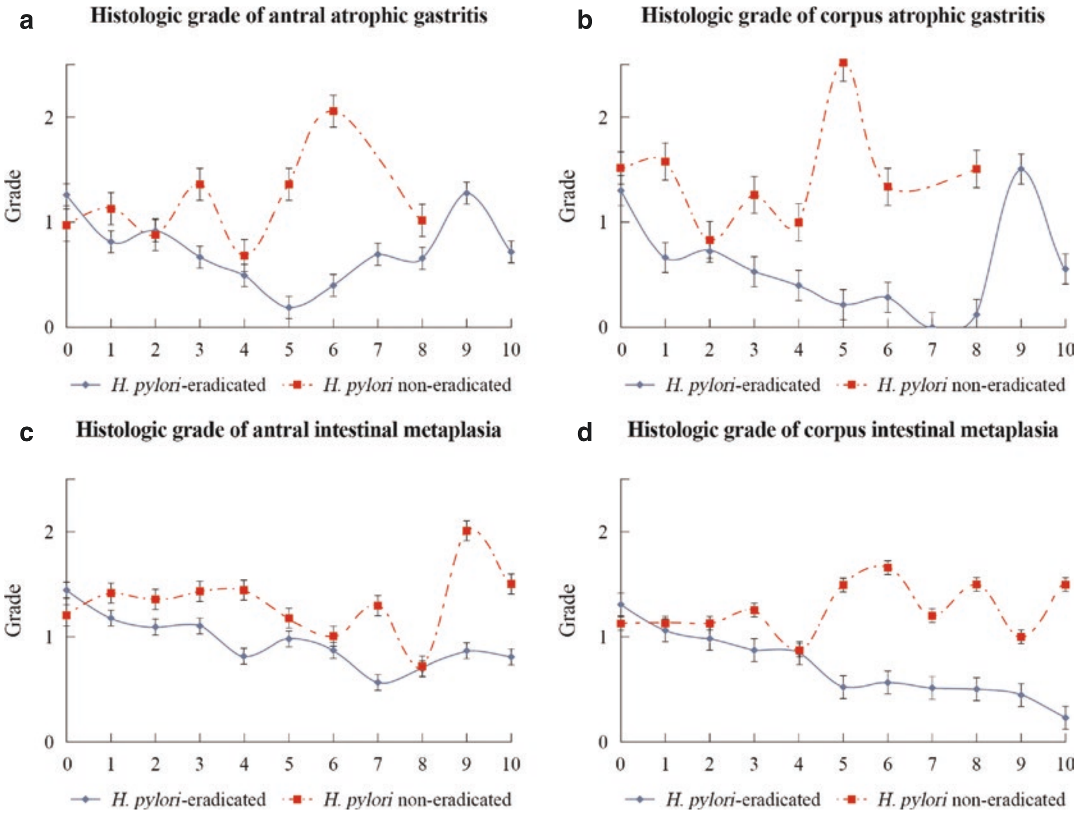

Fig. 55.2 Serial change of atrophic gastritis and intestinal metaplasia (IM). The histologic improvements of atrophy in the antrum (**a**) and corpus (**b**) by *H. pylori* eradication were more rapid than those of antral (**c**) and corpus IM (**d**) (Adapted from Hwang et al. [67])

55.4 Underlying of Reversibility of Atrophic Gastritis and Intestinal Metaplasia

As long-term follow-up studies showed the reversibility effect of IM, there must be a underlying mechanisms which takes long time after the *H. pylori* eradication. Maybe decrease of *CDX1* and *CDX2* gene and changing of gastric stem cells might contribute to these changes after *H. pylori* eradication.

55.4.1 CDX1 and CDX2

CDX1 and CDX2 are homeodomain transcription factors, localized specifically to the mucosa of the gut [68, 69]. CDX1 and CDX2 may play a crucial role in the development, maintenance, proliferation, and anterior–posterior patterning of the intestinal epithelium. While CDX2 is highly expressed in the small intestine and its expression declines when progressing distally in the colon, the opposite is observed for CDX1 [21]. Aberrant expression of CDX1 and CDX2 in gastric mucosae is related to *H. pylori* infection and IM, which might have a role in gastric carcinogenesis. In general, normal gastric mucosae do not express CDX1/CDX2, but aberrant expression of CDX1/CDX2 has been observed in animal and human gastric IM [70–72]. In some studies, expression of CDX2 has been reported to be positively correlated with dysplasia and cancer [22, 73, 74], but several studies have reported a reduction in CDX1/CDX2 in GC compared to IM [70, 72]. First of all our team evaluated the role of CDX1 and CDX2 in the formation of IM and the progression to dysplasia and GC. A total of 270 subjects included 90 with GC, dysplasia and age- and sex-matched controls. Real-time PCR (RT-PCR) was performed with corpus specimens for CDX1 and CDX2. The expression of CDX2 was significantly higher in *H. pylori*-positive group than *H. pylori*-negative group ($p = 0.045$) [22]. CDX1 and CDX2 expression increased proportional to the IM grade of the corpus ($p < 0.001$) (Fig. 55.3). CDX2 expression was significantly higher in incomplete type of IM than in complete type ($p = 0.045$) [22]. The expression of CDX1 in dysplasia group was significantly higher than in the control group ($p = 0.001$) [22], in addition, CDX1 and CDX2 in cancer group was significantly higher than control group ($p < 0.001$ and $p < 0.001$, respectively) [22]. Aberrant expression of CDX1 and CDX2 correlated with *H. pylori* infection and grade of IM in the corpus [22]. Furthermore, the results suggest that CDX1 and CDX2 play a role in the progression to GC and dysplasia [22].

In the next our team evaluated the changes in CDX1/CDX2 expression after *H. pylori* eradication, in relation to the reversibility of IM [21]. Time course of CDX1/CDX2 expressions was investigated in 176 subjects with various gastroduodenal disorders [21]. Among them, 132 patients were *H. pylori* positives; *H. pylori* were eradicated in 107 of them; 13 failed to eradicate; and 12 did not receive *H. pylori* eradication therapy. Forty-four subjects were *H. pylori* negatives. Expression levels in CDX1 and CDX2 from noncancerous gastric mucosae of the corpus, as well as the histologic findings of gastric mucosae, were evaluated during the follow-up [21]. Average follow-up duration was 33.7 months (range 2–97 months). Expression levels in both CDX1 and CDX2 mRNAs were correlated with IM grade in the corpus ($\rho = 0.633$ and 0.554, respectively, all $p < 0.001$) [21] (Fig. 55.4). Changes in CDX1/CDX2 mRNA expressions following *H. pylori* eradication showed only insignificant results; IM grade at the antrum and corpus showed a tendency to decrease after *H. pylori* eradication without statistical significance. However, histologic improvement of IM at the corpus was associated with a decrease in CDX2 mRNA expression during the follow-up (linear mixed model, p for slope = 0.015) [21] (Fig. 55.5). Changes in CDX2 mRNA expression levels during the follow-up according to the improvement or aggravation of IM grade at the corpus ($n = 176$). Histologic improvement of IM was associated with a decrease in CDX2 mRNA expression levels (a), while there were only insignificant results in the “IM undetermined” and

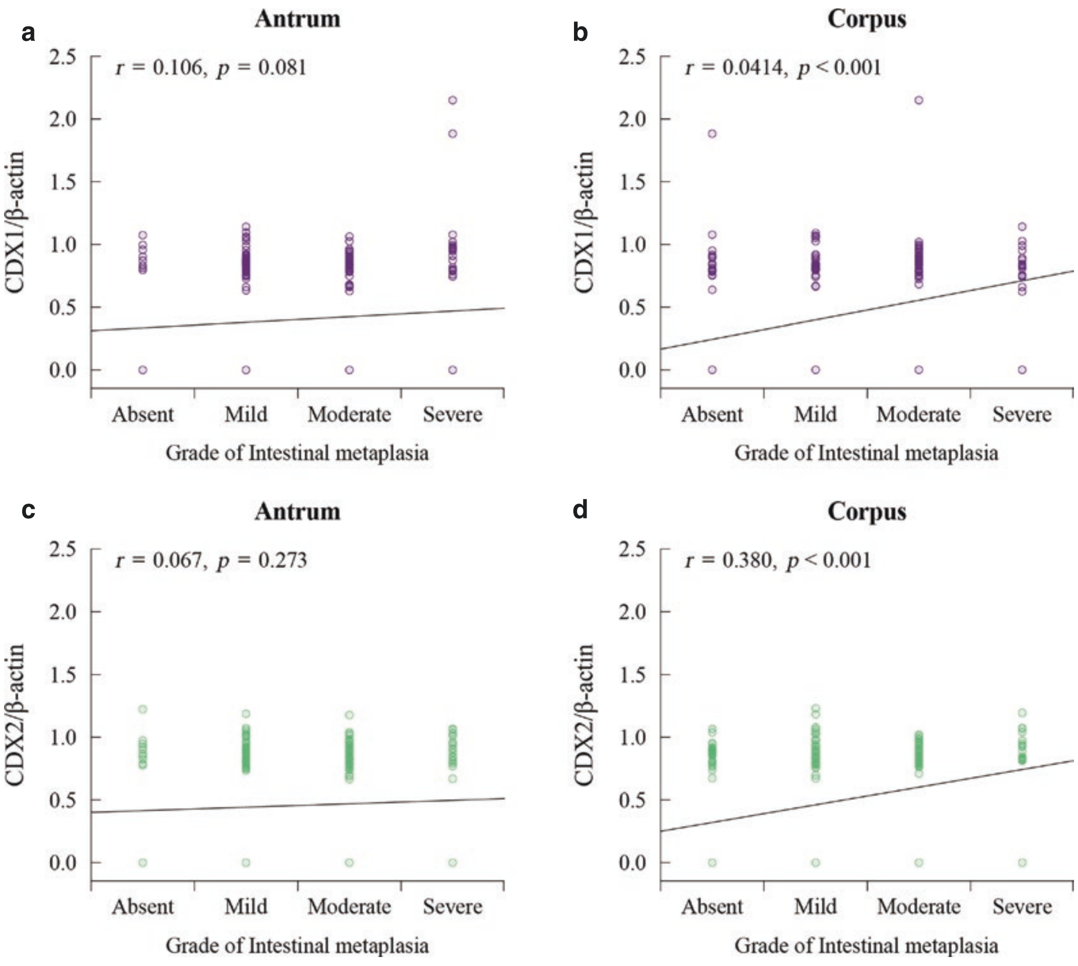

Fig. 55.3 Correlation between CDX1 and CDX2 expression and grade of intestinal metaplasia. CDX1 in the antrum (**a**) and corpus (**b**), CDX2 in the antrum (**c**) and corpus (**d**). *r* correlation coefficient (Adapted from Kang et al. [22])

“IM aggravated” groups (b, c) [21] (Fig. 55.6). These results suggest that the reversibility of IM may be associated with a decrease in CDX2 mRNA expression.

55.4.2 Gastric Stem Cells

The cancer stem cell (CSC) hypothesis that CSC may initiate carcinogenesis including IM that has emerged [75]. Some data suggest that CSCs are present in many solid tumors and cancer cells are derived from a CSC compartment which undergoes an abnormal replicative process to form a non-stem cell component of the tumor [76]. There has been growing evidence that *H. pylori* may promote emergence of CSCs in GC [77, 78]. The purification and characterization of CSCs could facilitate the identification of the marker for CSCs is the most important step. Unfortunately, CSCs of GC have not yet been defined and characterized. Various CSC markers for GC such as CD44, ALDH1, Leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), and CD166 were reported [79]. Although CD44 has been used most frequently as a marker for CSCs in GC, previous studies have suggested that CD44v8–10, variant form of CD44, was a specific maker for gastric CSCs, but not CD44s [80, 81]. LGR5 is an adult stem cell marker expressed in the small intestine, colon, stomach, and hair follicles [82]. Currently,

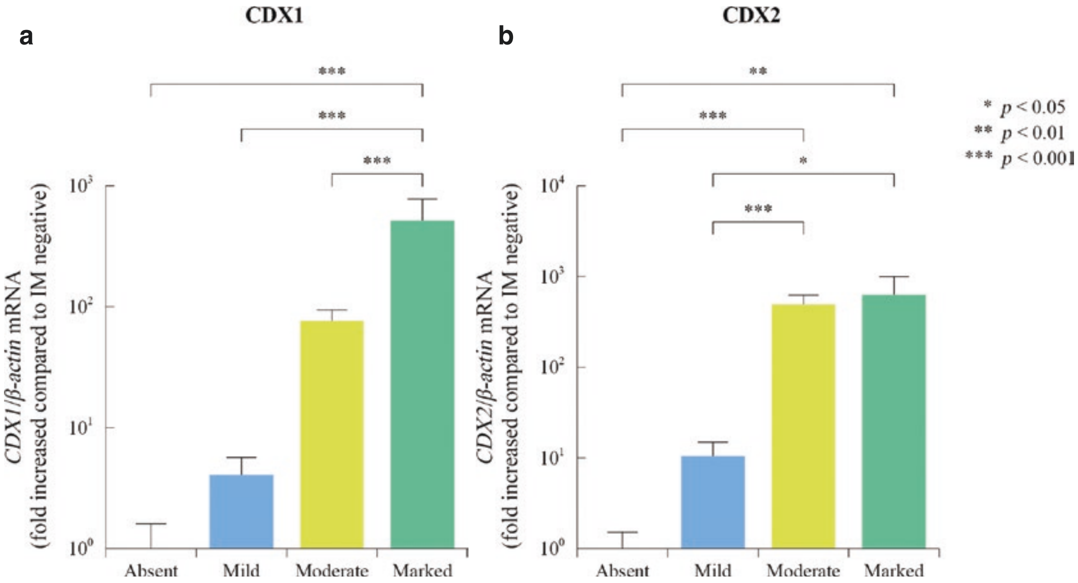

Fig. 55.4 Correlation between CDX1 (**a**) and CDX2 (**b**) mRNA expressions and the grade of intestinal metaplasia (IM) at the corpus (n = 176). Both CDX1 and CDX2 mRNA expression levels were significantly correlated with IM grade (Spearman's correlation coefficient ρ = 0.633 and 0.554, respectively, all $p < 0.001$.) p-values were calculated using one-way ANOVA with post-hoc HSD-Turkey test for pair-wise comparisons between groups. Data are presented as mean ± standard error (Adapted from Shin et al. [21])

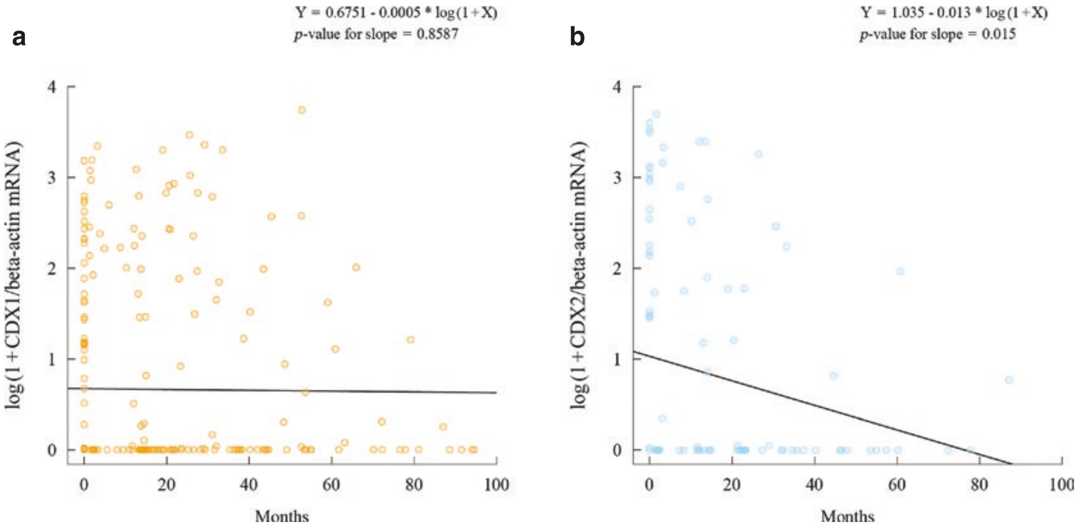

Fig. 55.5 Effects of *Helicobacter pylori* eradication on CDX1 (**a**) and CDX2 (**b**) mRNA expression levels (n = 107) (Adapted from Shin et al. [21])

in vivo lineage tracing revealed that LGR5-postive cells at the base of the pyloric glands were multipotent stem cells that contributed to daily epithelial renewal [83]. The technique of sorting cells based on cell surface marker expression and assessing their ability to initiate tumor growth in immunocompromised mice was a common method to investigate any possible existence of solid-organ CSCs. However, the clinical implication of measuring the expression

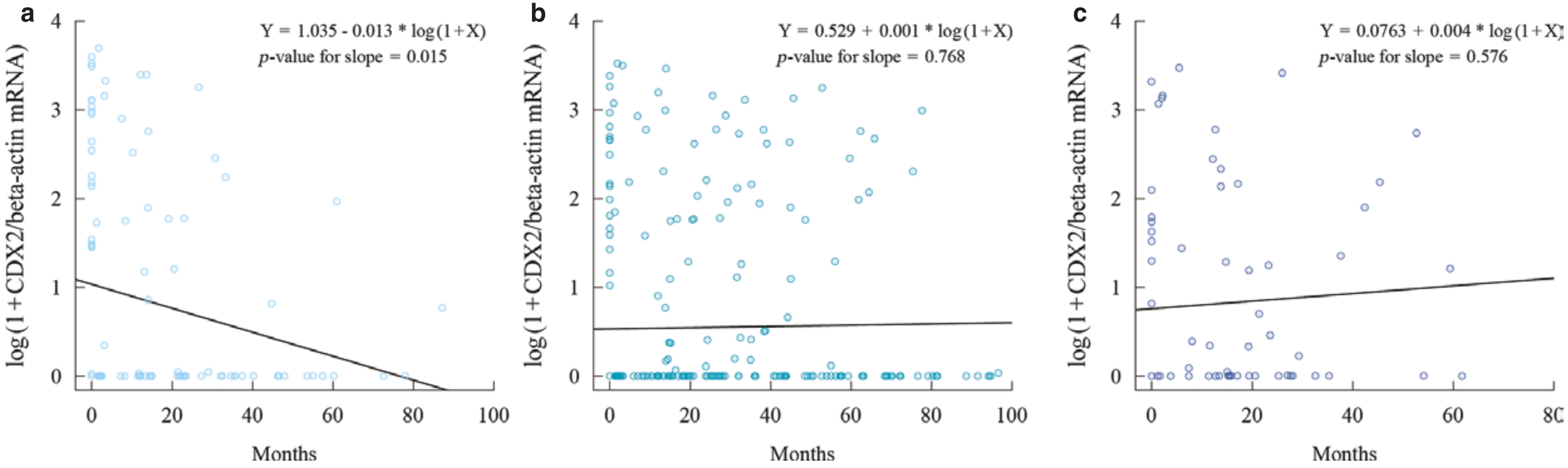

Fig. 55.6 Changes in CDX2 mRNA expression levels during the follow-up according to the improvement or aggravation of intestinal metaplasia (IM) grade at the corpus (n = 176). Histologic improvement of IM was associated with a decrease in CDX2 mRNA expression levels (**a**), while there were only insignificant results in the "IM undetermined" and "IM aggravated" groups (**b, c**) (Adapted from Shin et al. [21])

of CD44 and LGR5 in the human tissue has not been evaluated so far. Based on the above findings, we examined the potential application of evaluation of CD44 and LGR5 expression on human gastric mucosa in the normal-dysplasia-carcinoma sequence [75]. We compared the mRNA expression of LGR5, CD44 and CD44v8–10 and immunohistochemistry (IHC) of LGR5 and CD44 in gastric antral mucosa of 45 controls, 36 patients with gastric dysplasia, and 39 patients with EGC [75]. Additionally, IHC of LGR5 in gastric corpus mucosa was analysed [75]. Normal mucosa adjacent to dysplastic or cancer lesions was used for the quantitative RT-PCR and IHC. The immunoreactivity of LGR5 in the base of antral mucosa was higher in non-cancerous tissues of cancer than those of control ($p = 0.006$), whereas the expression of LGR5 mRNA was not different among the three groups [75] (Fig. 55.7). Immunostaining of LGR5 was much stronger in the antrum than in the corpus of stomach ($p < 0.001$) [75] (Fig. 55.8). Although there was no difference in the antral immunointensity of LGR5 according to the severity of IM, stronger immunostaining was found in the corpus with an aggravation of IM (p trend <0.001) [75] (Fig. 55.8). The expression of CD44v8–10 mRNA was higher in cancer patients than control subjects and patients with dysplasia ($p = 0.018$ and 0.009) while the expression of CD44 mRNA was higher in the control groups than the others [75] (Fig. 55.9). In addition, there was correlation between CD44 IHC score and LGR5 IHC score [75] (Fig. 55.10). These results suggest that IHC of LGR5 in crypt base and CD44 may be used for gastric CSC markers [75]. LGR5 expression may be associated with the developing of corpus IM. The expression of CD44v8–10 mRNA would be more suitable for GC stem cell marker than CD44 or LGR5 mRNA [75]. Maybe, these are valuable topics for the underlying mechanism of IM and its long time requiring of IM reversibility after the *H. pylori* eradication.

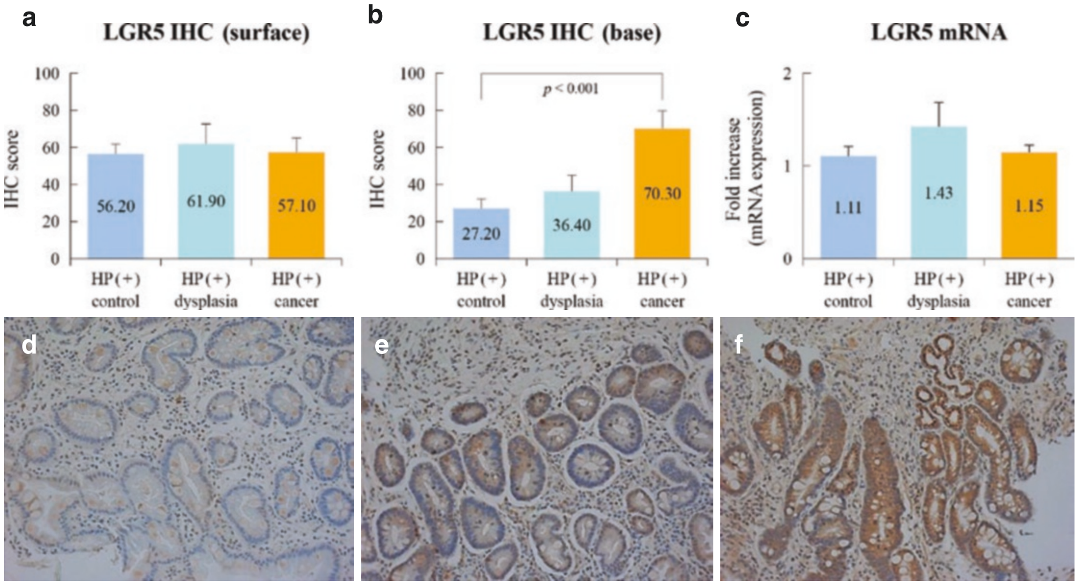

Fig. 55.7 LGR5 expression according to normal-dysplasia-cancer sequence. There was statistically significant difference in expression of neither surface LGR5 IHC (**a**), or LGR5 mRNA (**c**), while immunoreactivity of LGR5 (base) was higher in cancer than in control samples (**b**). LGR5 immunostaining in base of gastric mucosa in representative control (**d**), dysplasia (**e**) and cancer samples (**f**). Data shown are means ± standard errors (SE). *HP*, *H. pylori*; *IHC*, immunohistochemistry; *LGR5*, leucine-rich; repeat-containing G protein-coupled receptor 5 (Adapted from Choi et al. [75])

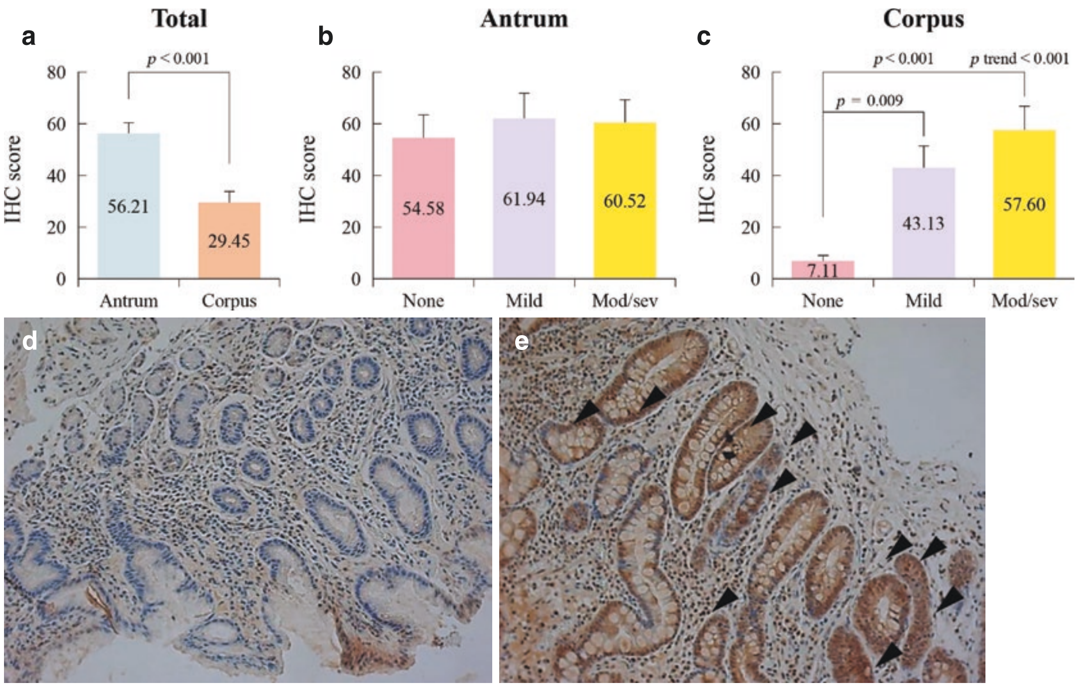

Fig. 55.8 Immunoreactivity of LGR5 according to the location of gastric mucosa and intestinal metaplasia (IM)**.** (**a**) LGR5 IHC in the corpus was significantly reduced than in the antrum. Antral mucosa (**b**) did not show any significant difference in LGR5 IHC according to presence or absence of IM. Gastric corpus mucosa with IM (**c**) showed an elevated immunointensity than in that without IM. The representative pictures of LGR 5 stating of basal part of gastric corpus mucosa without IM (**d**) and with severe IM (**e**). Data shown are means ± standard errors (SE) (Adapted from Choi et al. [75])

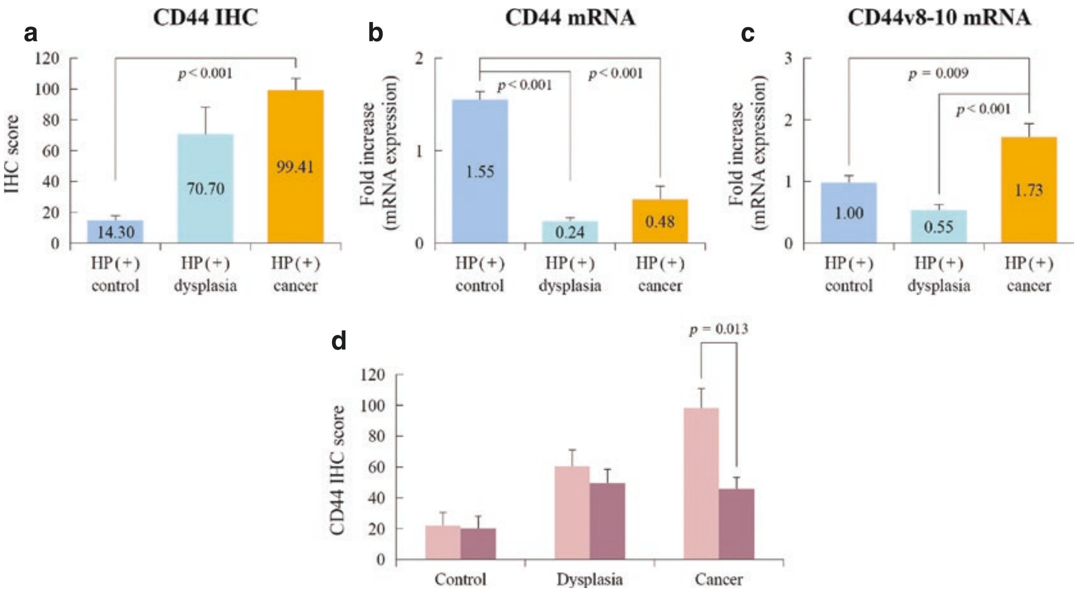

Fig. 55.9 CD44 expression according to normal-dysplasia-cancer sequence. Immunoreactivity of CD44 (**a**) was higher in cancer than in control samples. The expression of CD44s mRNA was higher in the control groups than the others (**b**) while that of CD44v8–10 mRNA was higher in cancer patients than control subjects and patients with dysplasia (p = 0.009 and < 0.001) (**c**). Immunoreactivity scores of CD44 (**d**) were analysed with a Wilcoxon rank sum test. The white bars indicate the initial score of immunostaining, whereas the black bars indicate staining at approximately 1 year after successful *H. pylori* eradication in *H. pylori*-positive subjects. *HP, H. pylori*; *IHC,* immunohistochemistry (Adapted from Choi et al. [75])

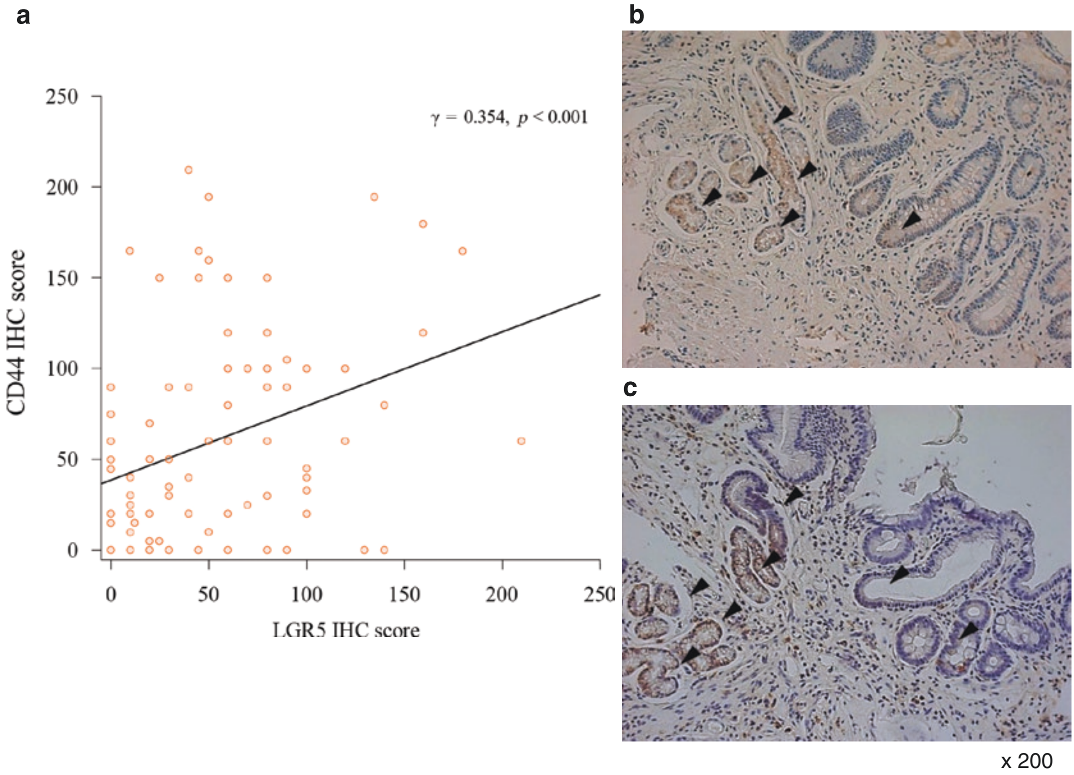

Fig. 55.10 Correlation between CD44 IHC score and LGR5 IHC score. (**a**) There was a significant positive correlation between of LGR5 and CD44 immunohistochemical results. The distribution of cells stained with (**b**) CD44 and (**c**) LGR5 were similar in serial sections (Adapted from Choi et al. [75])

55.5 Conclusions

Currently, many international guidelines recommend the *H. pylori* eradication in AG and IM. However, there are still many controversies in whether the *H. pylori* eradication improves AG and IM. Taking the results of related studies together, it seems likely that *H. pylori* eradication improves AG to some extent. However, a dominant opinion was that IM which progressed to a certain level is already an irreversible status (point of no return). Anyway, it is clear that the possibility of improvement of AG and IM is higher when *H. pylori* infection was treated in the early stage of these precancerous lesions than the advanced stage. Recently, long-term follow-up studies for the same cohort frequently showed the reversibility of AG and IM. The main mechanism of reversibility of IM may be associated with a decrease in the CDX2 mRNA expression. It is also evident that the decrease of AG and IM by the *H. pylori* eradication could be affected by other factors such as young age, high income, and *cagA*-positive status. Therefore, further in-depth studies regarding when is the optimal timing to treat *H. pylori* infection in patients with AG and IM are required.

References

1. Correa P. A human model of gastric carcinogenesis. Cancer Res. 1988;48:3554–60.
2. Wong BC, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, et al. *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. JAMA. 2004;291:187–94.
3. Maconi G, Lazzaroni M, Sangaletti O, Bargiggia S, Vago L, BianchiPorro G. Effect of *Helicobacter pylori* eradication on gastric histology, serum gastrin and pepsinogen I levels, and gastric emptying

in patients with gastric ulcer. Am J Gastroenterol. 1997;92:1844–8.

4. Sung JJ, Lin SR, Ching JY, Zhou LY, To KF, Wang RT, et al. Atrophy and intestinal metaplasia one year after cure of *H. Pylori* infection: a prospective, randomized study. Gastroenterology. 2000;119:7–14.
5. Leung WK, Lin SR, Ching JY, To KF, Ng EK, Chan FK, et al. Factors predicting progression of gastric intestinal metaplasia: results of a randomised trial on *Helicobacter pylori* eradication. Gut. 2004;53:1244–9.
6. Kodama M, Okimoto T, Ogawa R, Mizukami K, Murakami K. Endoscopic atrophic classification before and after *H. Pylori* eradication is closely associated with histological atrophy and intestinal metaplasia. Endosc Int Open. 2015;3:E311–7.
7. Lu B, Chen MT, Fan YH, Liu Y, Meng LN. Effects of *Helicobacter pylori* eradication on atrophic gastritis and intestinal metaplasia: a 3-year follow-up study. World J Gastroenterol. 2005;11:6518–20.
8. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of *Helicobacter pylori* infection-the Maastricht IV/Florence consensus report. Gut. 2012;61:646–64.
9. Zhou L, Sung JJ, Lin S, Jin Z, Ding S, Huang X, et al. A five-year follow-up study on the pathological changes of gastric mucosa after *H pylori* eradication. Chin Med J. 2003;116:11–4.
10. Cho SJ, Choi IJ, Kook MC, Yoon H, Park S, Kim CG, et al. Randomised clinical trial: the effects of *Helicobacter pylori* eradication on glandular atrophy and intestinal metaplasia after subtotal gastrectomy for gastric cancer. Aliment Pharmacol Ther. 2013;38:477–89.
11. Kiriyama Y, Tahara T, Shibata T, Okubo M, Nakagawa M, Okabe A, et al. Gastric-and-intestinal mixed intestinal metaplasia is irreversible point with eradication of *Helicobacter pylori*. Open J Pathol. 2016;6:93.
12. Kang JM, Kim N, Shin CM, Lee HS, Lee DH, Jung HC, et al. Predictive factors for improvement of atrophic gastritis and intestinal metaplasia after *Helicobacter pylori* eradication: a three-year follow-up study in Korea. Helicobacter. 2012;17:86–95.
13. Lahner E, Bordi C, Cattaruzza MS, Iannoni C, Milione M, Delle Fave G, et al. Long-term follow-up in atrophic body gastritis patients: atrophy and intestinal metaplasia are persistent lesions irrespective of *Helicobacter pylori* infection. Aliment Pharmacol Ther. 2005;22:471–81.
14. Hwang YJ, Kim N, Yun CY, Kwon MG, Baek SM, Kwon YJ, et al. Predictive factors for improvement of atrophic gastritis and intestinal metaplasia: A long-term prospective clinical study. Korean J Helicobacter Up Gastrointest Res. 2018;18:186–97.
15. Wu CY, Kuo KN, Wu MS, Chen YJ, Wang CB, Lin JT. Early *Helicobacter pylori* eradication decreases risk of gastric cancer in patients with peptic ulcer disease. Gastroenterology. 2009;137:1641–8.e1-2.
16. Hwang YJ, Kim N, Lee HS, Lee JB, Choi YJ, Yoon H, et al. Reversibility of atrophic gastritis and intestinal metaplasia after *Helicobacter pylori* eradication—a prospective study for up to 10 years. Aliment Pharmacol Ther. 2018;47:380–90.
17. Chiang TH, Chang WJ, Chen SL, Yen AM, Fann JC, Chiu SY, et al. Mass eradication of *Helicobacter pylori* to reduce gastric cancer incidence and mortality: a long-term cohort study on Matsu Islands. Gut. 2021;70:243–50.
18. Yoon K, Kim N. Reversibility of atrophic gastritis and intestinal metaplasia by eradication of *Helicobacter pylori*. Korean J Gastroenterol. 2018;72:104–15.
19. Yoon K, Kim N. Significance of *Helicobacter pylori* eradication on atrophic gastritis and intestinal metaplasia. Korean J Helicobacter Up Gastrointest Res. 2020;20:107–16.
20. Kwon YJ, Kim N, Baek SM, Lee HS, Hwang YJ, Lee J, et al. Prevalence of histologic atrophic gastritis and intestinal metaplasia in the corpus has decreased over 15 years in females in the Korean population. Helicobacter. 2019;24:e12579.
21. Shin CM, Kim N, Chang H, Kim JS, Lee DH, Jung HC. Follow-up study on CDX1 and CDX2 mRNA expression in noncancerous gastric mucosae after *Helicobacter pylori* eradication. Dig Dis Sci. 2016;61:1051–9.
22. Kang JM, Lee BH, Kim N, Lee HS, Lee HE, Park JH, et al. CDX1 and CDX2 expression in intestinal metaplasia, dysplasia and gastric cancer. J Korean Med Sci. 2011;26:647–53.
23. Kim N, Lim SH, Lee KH, Choi SE, Jung HC, Song IS, et al. Long-term effects of *Helicobacter pylori* eradication on intestinal metaplasia in patients with duodenal and benign gastric ulcers. Dig Dis Sci. 2000;45:1754–62.
24. Annibale B, Aprile MR, D'Ambra G, Caruana P, Bordi C, Delle FG. Cure of *Helicobacter pylori* infection in atrophic body gastritis patients does not improve mucosal atrophy but reduces hypergastrinemia and its related effects on body ECL-cell hyperplasia. Aliment Pharmacol Ther. 2000;14:625–34.
25. Ohkusa T, Fujiki K, Takashimizu I, Kumagai J, Tanizawa T, Eishi Y, et al. Improvement in atrophic gastritis and intestinal metaplasia in patients in whom *Helicobacter pylori* was eradicated. Ann Intern Med. 2001;134:380–6.
26. Ruiz B, Garay J, Correa P, Fontham ET, Bravo JC, Bravo LE, et al. Morphometric evaluation of gastric antral atrophy: improvement after cure of *Helicobacter pylori* infection. Am J Gastroenterol. 2001;96:3281–7.
27. Ito M, Haruma K, Kamada T, Mihara M, Kim S, Kitadai Y, et al. *Helicobacter pylori* eradication therapy improves atrophic gastritis and intestinal metaplasia: a 5-year prospective study of patients with atrophic gastritis. Aliment Pharmacol Ther. 2002;16:1449–56.
28. Annibale B, Di Giulio E, Caruana P, Lahner E, Capurso G, Bordi C, et al. The long-term effects of cure of *Helicobacter pylori* infection on patients with atrophic body gastritis. Aliment Pharmacol Ther. 2002;16:1723–31.

29. Yamada T, Miwa H, Fujino T, Hirai S, Yokoyama T, Sato N. Improvement of gastric atrophy after *Helicobacter pylori* eradication therapy. J Clin Gastroenterol. 2003;36:405–10.
30. Iacopini F, Consolazio A, Bosco D, Marcheggiano A, Bella A, Pica R, et al. Oxidative damage of the gastric mucosa in *Helicobacter pylori* positive chronic atrophic and nonatrophic gastritis, before and after eradication. Helicobacter. 2003;8:503–12.
31. Kamada T, Haruma K, Hata J, Kusunoki H, Sasaki A, Ito M, et al. The long-term effect of *Helicobacter pylori* eradication therapy on symptoms in dyspeptic patients with fundic atrophic gastritis. Aliment Pharmacol Ther. 2003;18:245–52.
32. Wambura C, Aoyama N, Shirasaka D, Kuroda K, Maekawa S, Ebara S, et al. Influence of gastritis on cyclooxygenase-2 expression before and after eradication of *Helicobacter pylori* infection. Eur J Gastroenterol Hepatol. 2004;16:969–79.
33. Kamada T, Hata J, Sugiu K, Kusunoki H, Ito M, Tanaka S, et al. Clinical features of gastric cancer discovered after successful eradication of *Helicobacter pylori*: results from a 9-year prospective follow-up study in Japan. Aliment Pharmacol Ther. 2005;21:1121–6.
34. Toyokawa T, Suwaki K, Miyake Y, Nakatsu M, Ando M. Eradication of *Helicobacter pylori* infection improved gastric mucosal atrophy and prevented progression of intestinal metaplasia, especially in the elderly population: a long-term prospective cohort study. J Gastroenterol Hepatol. 2010;25:544–7.
35. Park YH, Kim N. Review of atrophic gastritis and intestinal metaplasia as a premalignant lesion of gastric cancer. J Cancer Prev. 2015;20:25–40.
36. Mera R, Fontham ET, Bravo LE, Bravo JC, Piazuelo MB, Camargo MC, et al. Long term follow up of patients treated for *Helicobacter pylori* infection. Gut. 2005;54:1536–40.
37. Correa P, Fontham ET, Bravo JC, Bravo LE, Ruiz B, Zarama G, et al. Chemoprevention of gastric dysplasia: randomized trial of antioxidant supplements and anti-*Helicobacter pylori* therapy. J Natl Cancer Inst. 2000;92:1881–8.
38. You WC, Brown LM, Zhang L, Li JY, Jin ML, Chang YS, et al. Randomized double-blind factorial trial of three treatments to reduce the prevalence of precancerous gastric lesions. J Natl Cancer Inst. 2006;98:974–83.
39. Lee YC, Chen TH, Chiu HM, Shun CT, Chiang H, Liu TY, et al. The benefit of mass eradication of *Helicobacter pylori* infection: a community-based study of gastric cancer prevention. Gut. 2013;62:676–82.
40. Yan L, Chen Y, Chen F, Tao T, Hu Z, Wang J, et al. Effect of *Helicobacter pylori* eradication on gastric cancer prevention: updated report from a randomized controlled trial with 26.5 years of follow-up. Gastroenterology. 2022;163(154–162):e3.
41. Choi IJ, Kim CG, Lee JY, Kim YI, Kook MC, Park B, et al. Family history of gastric cancer and *Helicobacter pylori* treatment. N Engl J Med. 2020;382:427–36.
42. Shiotani A, Haruma K, Graham DY. Metachronous gastric cancer after successful *Helicobacter pylori* eradication. World J Gastroenterol. 2014;20:11552–9.
43. Asaka M. A new approach for elimination of gastric cancer deaths in Japan. Int J Cancer. 2013;132:1272–6.
44. Leung WK, Wong IOL, Cheung KS, Yeung KF, Chan EW, Wong AYS, et al. Effects of *Helicobacter pylori* eradication on the incidence of gastric cancer in older individuals. Gastroenterology. 2018;155:67–75.
45. Uemura N, Mukai T, Okamoto S, Yamaguchi S, Mashiba H, Taniyama K, et al. Effect of *Helicobacter pylori* eradication on subsequent development of cancer after endoscopic resection of early gastric cancer. Cancer Epidemiol Biomark Prev. 1997;6:639–42.
46. Fukase K, Kato M, Kikuchi S, Inoue K, Uemura N, Okamoto S, et al. Effect of eradication of *Helicobacter pylori* on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. Lancet. 2008;372:392–7.
47. Maehata Y, Nakamura S, Fujisawa K, Esaki M, Moriyama T, Asano K, et al. Long-term effect of *Helicobacter pylori* eradication on the development of metachronous gastric cancer after endoscopic resection of early gastric cancer. Gastrointest Endosc. 2012;75:39–46.
48. Choi J, Kim SG, Yoon H, Im JP, Kim JS, Kim WH, et al. Eradication of *Helicobacter pylori* after endoscopic resection of gastric tumors does not reduce incidence of metachronous gastric carcinoma. Clin Gastroenterol Hepatol. 2014;12:793–800.
49. Bae SE, Jung HY, Kang J, Park YS, Baek S, Jung JH, et al. Effect of *Helicobacter pylori* eradication on metachronous recurrence after endoscopic resection of gastric neoplasm. Am J Gastroenterol. 2014;109:60–7.
50. Choi IJ, Kook MC, Kim YI, Cho SJ, Lee JY, Kim CG, et al. *Helicobacter pylori* therapy for the prevention of metachronous gastric cancer. N Engl J Med. 2018;378:1085–9.
51. Yoon SB, Park JM, Lim CH, Cho YK, Choi MG. Effect of *Helicobacter pylori* eradication on metachronous gastric cancer after endoscopic resection of gastric tumors: a meta-analysis. Helicobacter. 2014;19:243–8.
52. Lin YS, Chen MJ, Shih SC, Bair MJ, Fang CJ, Wang HY. Management of *Helicobacter pylori* infection after gastric surgery. World J Gastroenterol. 2014;20:5274–82.
53. Rokkas T, Pistiolas D, Sechopoulos P, Robotis I, Margantinis G. The long-term impact of *Helicobacter pylori* eradication on gastric histology: a systematic review and meta-analysis. Helicobacter. 2007;12(Suppl 2):32–8.
54. Wang J, Xu L, Shi R, Huang X, Li SW, Huang Z, et al. Gastric atrophy and intestinal metaplasia before and after *Helicobacter pylori* eradication: a meta-analysis. Digestion. 2011;83:253–60.
55. Kong YJ, Yi HG, Dai JC, Wei MX. Histological changes of gastric mucosa after *Helicobacter pylori* eradication: a systematic review and meta-analysis. World J Gastroenterol. 2014;20:5903–11.

56. Dinis-Ribeiro M, Areia M, de Vries AC, Marcos-Pinto R, Monteiro-Soares M, O'Connor A, et al. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European *Helicobacter* study group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). Endoscopy. 2012;44:74–94.
57. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection—the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
58. Kim SG, Jung H-K, Lee HL, Jang JY, Lee H, Kim CG, et al. Guidelines for the diagnosis and treatment of *Helicobacter pylori* infection in Korea, 2013 revised edition. Korean J Gastroenterol. 2013;62:3–26.
59. Liu WZ, Xie Y, Cheng H, Lu NH, Hu FL, et al. Fourth Chinese National Consensus Report on the management of *Helicobacter pylori* infection. J Dig Dis. 2013;14:211–21.
60. Jung HK, Kang SJ, Lee YC, Yang HJ, Park SY, Shin CM, et al. Evidence-based guidelines for the treatment of *Helicobacter pylori* infection in Korea 2020. Gut Liver. 2021;15:168–95.
61. Kuipers EJ. Review article: relationship between *Helicobacter pylori*, atrophic gastritis and gastric cancer. Aliment Pharmacol Ther. 1998;12(Suppl 1):25–36.
62. Lim JH, Kim N, Lee HS, Choe G, Jo SY, Chon I, et al. Correlation between endoscopic and histological diagnoses of gastric intestinal metaplasia. Gut Liver. 2013;7:41–50.
63. Lee SY. Current progress toward eradicating *Helicobacter pylori* in east Asian countries: differences in the 2013 revised guidelines between China, Japan, and South Korea. World J Gastroenterol. 2014;20:1493–502.
64. Take S, Mizuno M, Ishiki K, Yoshida T, Ohara N, Yokota K, et al. The long-term risk of gastric cancer after the successful eradication of *Helicobacter pylori*. J Gastroenterol. 2011;46:318–24.
65. Lee SY. Future candidates for indications of *Helicobacter pylori* eradication: do the indications need to be revised? J Gastroenterol Hepatol. 2012;27:200–11.
66. Lee SY. New guidelines for *Helicobacter pylori* treatment: comparisons between Korea and Japan. Korean J Gastroenterol. 2014;63:151–7.
67. Hwang YJ, Choi Y, Kim N, Lee HS, Yoon H, Shin CM, et al. The difference of endoscopic and histologic improvements of atrophic gastritis and intestinal metaplasia after *Helicobacter pylori* eradication. Dig Dis Sci. 2022;67:3055–66.
68. Silberg DG, Swain GP, Suh ER, Traber PG. Cdx1 and cdx2 expression during intestinal development. Gastroenterology. 2000;119:961–71.
69. Suh E, Chen L, Taylor J, Traber PG. A homeodomain protein related to caudal regulates intestine-specific gene transcription. Mol Cell Biol. 1994;14:7340–51.
70. Silberg DG, Furth EE, Taylor JK, Schuck T, Chiou T, Traber PG. CDX1 protein expression in normal, metaplastic, and neoplastic human alimentary tract epithelium. Gastroenterology. 1997;113:478–86.
71. Silberg DG, Sullivan J, Kang E, Swain GP, Moffett J, Sund NJ, et al. Cdx2 ectopic expression induces gastric intestinal metaplasia in transgenic mice. Gastroenterology. 2002;122:689–96.
72. Almeida R, Silva E, Santos-Silva F, Silberg DG, Wang J, De Bolós C, et al. Expression of intestine-specific transcription factors, CDX1 and CDX2, in intestinal metaplasia and gastric carcinomas. J Pathol. 2003;199:36–40.
73. Seno H, Oshima M, Taniguchi MA, Usami K, Ishikawa TO, Chiba T, et al. CDX2 expression in the stomach with intestinal metaplasia and intestinal-type cancer: prognostic implications. Int J Oncol. 2002;21:769–74.
74. Kim HS, Lee JS, Freund JN, Min KW, Lee JS, Kim W, et al. CDX-2 homeobox gene expression in human gastric carcinoma and precursor lesions. J Gastroenterol Hepatol. 2006;21:438–42.
75. Choi YJ, Kim N, Lee HS, Park SM, Park JH, Yoon H, et al. Expression of leucine-rich repeat-containing G-protein coupled receptor 5 and CD44: Potential implications for gastric cancer stem cell marker. J Cancer Prev. 2016;21:279–87.
76. Takaishi S, Okumura T, Wang TC. Gastric cancer stem cells. J Clin Oncol. 2008;26:2876–82.
77. Choi YJ, Kim N, Chang H, Lee HS, Park SM, Park JH, et al. *Helicobacter pylori*-induced epithelial-mesenchymal transition, a potential role of gastric cancer initiation and an emergence of stem cells. Carcinogenesis. 2015;36:553–63.
78. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. 2008;133:704–15.
79. Levi E, Sochacki P, Khoury N, Patel BB, Majumdar AP. Cancer stem cells in *Helicobacter pylori* infection and aging: implications for gastric carcinogenesis. World J Gastrointest Pathophysiol. 2014;5:366–72.
80. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008;3:1101–8.
81. Lau WM, Teng E, Chong HS, Lopez KA, Tay AY, Salto-Tellez M, et al. CD44v8-10 is a cancer-specific marker for gastric cancer stem cells. Cancer Res. 2014;74:2630–41.
82. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature. 2007;449:1003–7.
83. Sigal M, Rothenberg ME, Logan CY, Lee JY, Honaker RW, Cooper RL, et al. *Helicobacter pylori* activates and expands Lgr5(+) stem cells through direct colonization of the gastric glands. Gastroenterology. 2015;148:1392–404.e21.

Primary Prevention of Gastric Cancer

56

Nayoung Kim

Abstract

As gastric cancer (GC) develops only in 1–3% of *Helicobacter pylori* (*H. pylori*)-infected population and it takes a very long time from *H. pylori* infection to gastric carcinogenesis, it is very difficult to prospectively demonstrate the effect of *H. pylori* eradication on the incidence of GC. Recently, several reports suggested a survival improvement after *H. pylori* eradication in large cohort of subjects. Especially, an analysis of data from a public hospital database suggested a benefit of survival by *H. pylori* eradication therapy in older subjects (60 years or older) who are the highest risk group, 10 or more years after treatment. Similarly, the individual studies and meta-analyses to date suggest that *H. pylori* eradication can reduce the relative risk for GC to approximately two-thirds. Guidelines all over the world consistently recommend *H. pylori* eradication as an effective strategy for the prevention of GC. Even it remains rather unclear how *H. pylori* eradication prevents GC the possible underlying mechanisms include reversibility of atrophic gastritis or intestinal metaplasia, methylation, transforming growth factor-β1 (TGF-β1) and TGF-β1-induced epithelial-mesenchymal transition and stem cells, and the positive effect of *H. pylori* eradication on the gastric microbiota.

Keywords

Gastric cancer · *Helicobacter pylori*
Eradication · Primary · Prevention · Atrophy
Intestinal metaplasia · Methylation
Epithelial-mesenchymal transition

56.1 Introduction

Considering that *Helicobacter pylori* (*H. pylori*) is the main cause of gastric cancer (GC), there are no controversies in that the ultimate goal of *H. pylori* eradication is the prevention of GC. The cascade of GC, a leading cause of cancer incidence and mortality, is multifactorial, but it is certain that *H. pylori* infection plays a major role in this cascade. However, GC develops only in a very small portion of *H. pylori*-infected population, and it takes a very long time from *H. pylori* infection to the development of GC. Therefore, it is very difficult to prospectively demonstrate the effect of *H. pylori* eradication on the incidence of GC [1]. Recently, there were several important publication which suggest a survival improvement after *H. pylori* eradication in large cohort of subjects [2, 3]. Especially, an analysis of data

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from a public hospital database suggested a benefit of survival by *H. pylori* eradication therapy in older subjects (60 years or older) who are the highest risk group, 10 or more years after treatment [2].

The Kyoto consensus report on gastritis designated *H. pylori* gastritis as an infectious diseases and it was accepted in the new International Statistical Classification of Disease 11th Revision (ICD-11β). It declared that all *H. pylori* positive subjects should receive eradication [4]. In this chapter, the evidences for the primary prevention effect of *H. pylori* eradication on the GC development were explored.

56.2 Key Individual Studies

Even though there are some controversies whether *H. pylori* eradication can improve atrophic gastritis (AG) and intestinal metaplasia (IM), there is no doubt that *H. pylori* eradication can prevent GC [5]. A series of patient-control studies which were published in 1990s reported that the risk of GC is three- to six-folds higher in the *H. pylori*-infected subjects compared with normal control [6–8]. In addition, there was a definite correlation between *H. pylori* infection and incidence of GC [6–8]. However, to make a definite conclusion regarding whether there is causality between *H. pylori* infection and development of GC and whether *H. pylori* eradication could decrease incidence of GC, large randomized controlled trials (RCTs) are required. Although it is very difficult to perform these studies, there are many studies showing that *H. pylori* eradication can decrease the incidence of GC.

Looking at the key individual studies, first, Uemura et al. checked the *H. pylori* infection status in Japanese people and followed them up for mean 8 years; they found that GC developed only in *H. pylori*-infected subjects [9]. Thereafter, the famous study of Wong et al. randomized 1630 *H. pylori*-infected healthy adults in an area of high risk of GC in China into placebo-administrated group and *H. pylori*-eradicated group and followed them up for mean 7.5 years. In the subgroup which has no precancerous lesions such as AG and IM, the incidence of GC significantly decreased in the *H. pylori*-eradicated group than in the placebo group [10]. Recently, the authors of this study updated the follow-up study [11]. They showed that during 26.5 years of follow-up, 21 participants (2.57%) in the treatment arm and 35 (4.31%) in the placebo arm were diagnosed with GC [11]. Participants receiving *H. pylori* treatment had a lower incidence of GC compared with their placebo counterparts (hazard ratio [HR] 0.57; 95% confidence interval [CI], 0.33–0.98) [11]. More obvious risk reduction was observed among those without premalignant gastric lesions (HR 0.37; 95% CI, 0.15–0.95) and those without dyspepsia symptoms at baseline (HR 0.44; 95% CI, 0.21–0.94) [11]. Furthermore, compared with 32 cases of GC observed among 527 participants with persistent *H. pylori* infection in the placebo group, only 16 were identified in 625 subjects with successful eradication in the treatment group (HR 0.46; 95% CI, 0.26–0.83) [11]. However, there were no statistically significant differences for any mortality end points between the two groups so far [11].

When Takenaka et al. performed *H. pylori* eradication in Japanese people in the year 1807, they found that the incidence of GC became significantly lower in the *H. pylori*-eradicated group than in the *H. pylori* non-eradicated group [12]. This protective effect of *H. pylori* eradication was especially remarkable for GC of intestinal type [12]. Another Japanese group made *H. pylori*-infected subjects decide to receive eradication therapy or not by themselves and followed them up for mean 3 years [13]. The authors reported that the cumulative incidence of GC became lower in the group who underwent *H. pylori* eradication than in the group who did not receive *H. pylori* eradication (relative risk [RR] 0.335) [13]. Again, this preventive effect of *H. pylori* eradication was significant only for differentiated GC [13]. The results of the observational study in the Matsu Islands of Taiwan were reported [14]. The authors treated *H. pylori*-infected subjects among the general population in 2004 and followed them up for 4 years [14]. The incidence of 5-year mean incidence of GC in the Matsu Islands decreased to approximately 25% (from 40.3/1000 person-year to 30.4/1000 person-year) as along with the decrease of *H. pylori* prevalence [14]. However, the incidence of GC in the mainland of Taiwan did not significantly change [14]. When they prolonged the follow-up period up to 2018, the presence and severity of AG and IM also decreased with time [15]. Compared with the historical control period from the year 1995 to 2003, the effectiveness in reducing GC incidence and mortality during the chemoprevention period was 53% (95% CI, 30–69%; $p < 0.001$) and 25% (95% CI, 14–51%; $p = 0.18$), respectively [15]. They concluded that a significant reduction in mortality is likely to be achieved with a longer follow-up period [15].

In Korea, there was a nice report that *H. pylori* eradication treatment reduced the risk of GC among persons with *H. pylori* infection who had a family history of GC in first-degree relatives [16]. In detail, a total of 1676 participants were included in the modified intention-to-treat population for the analysis of the primary outcome (832 in the treatment group and 844 in the placebo group) [16]. During a median follow-up of 9.2 years, GC developed in 10 participants (1.2%) in the treatment group and in 23 (2.7%) in the placebo group (HR 0.45; 95% CI, 0.21–0.94; $p = 0.03$ by log-rank test). Among the 10 participants in the treatment group in whom GC developed, five (50.0%) had persistent *H. pylori* infection. Thus, GC developed in 0.8% of participants (5 of 608) in whom *H. pylori* infection was eradicated but in 2.9% of participants (28 of 979) who had persistent infection (HR 0.27; 95% CI, 0.10–0.70) [16].

Usually, the benefit of *H. pylori* eradication treatment on the prevention effect of GC is known to be larger in younger people because the statistical significance appeared in the absence of AG and IM. Actually, AG and IM developed after chronic *H. pylori* infection; thus, they proportionally increased as the people became older [17, 18]. Usually, the preventive effect of the *H. pylori* eradication of GC was estimated to be higher in young population below 50 years of age. However, there was a very nice study analysis of data from a public hospital database, which clearly showed a benefit of survival by *H. pylori* eradication therapy in older subjects (60 years or older) who are the highest risk group of GC. That is, among 73,237 subjects infected with *H. pylori* who received eradication therapy, 200 (0.27%) developed GC during a median follow-up time of 7.6 years [2]. Compared with the matched general population, the GC risk was significantly decreased [2] (Fig. 56.1a). In the subgroup analysis, it was lower in subjects 60 years or older who had received *H. pylori* treatment (standardized incidence ratio [SIR] 0.82; 95% CI, 0.69–0.97; $p = 0.02$) but not in younger groups [2] (Fig. 56.1b). In addition, when data were stratified based on time from *H. pylori* treatment (less than 5 years, 5–9 years, and 10 or more years), the risk of GC was significantly lower than the general population 10 or more years after eradication in the group 40–59 years of age (SIR 0.32; 95% CI, 0.08–0.88; $p = 0.04$) and the group 60 years or older (SIR 0.42; 95% CI, 0.42–0.84; $p = 0.02$) than the other age groups [2]. It suggested that the eradication of *H. pylori* decreased the risk of GC, particularly in older subjects, 10 or more years after treatment [2]. Although the statistical significance in the older age group was originated because the incidence of GC was higher in the aged group but these results indicate that *H. pylori* eradication is useful even in the in older subjects (60 years or older).

These results justify the higher rate of *H. pylori* eradication therapy over 50 years in South Korea, in which the rate *H. pylori* eradication therapy increased as getting old [19] (Fig. 56.2). In detail, a national survey showed that 5563 (23.5%, 5563/23,632) reported a history of having received *H. pylori* eradication therapy, regardless of the outcome of therapy [19]. The rate of *H. pylori* eradication therapy rapidly increased with increasing age, except in two age groups (16–19 and 20–29 years) [19] (Fig. 56.2). Thus, the clinicians need to encourage *H. pylori* eradication therapy even the subjects are old.

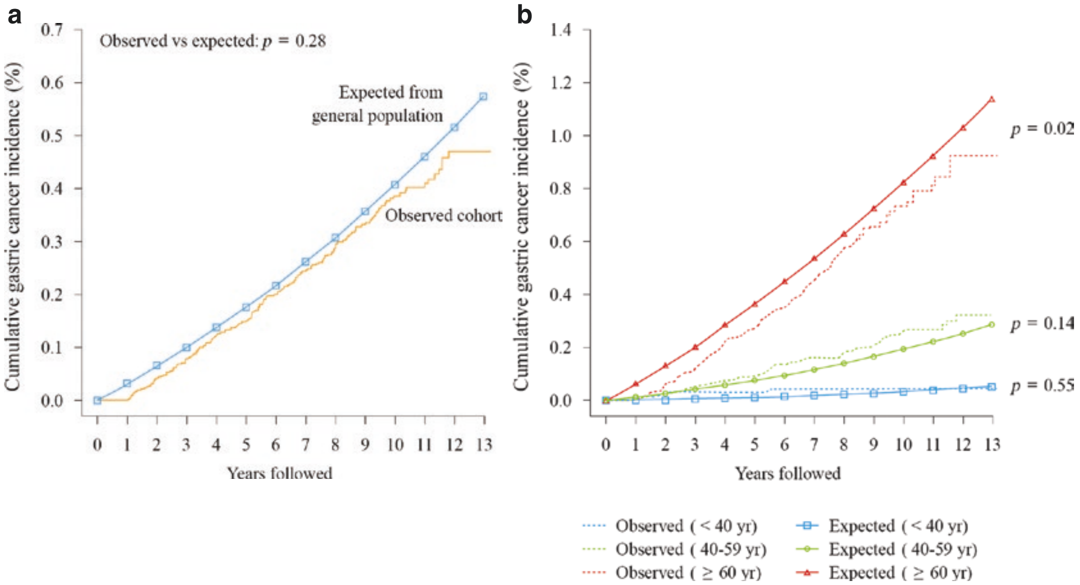

Fig. 56.1 Cumulative gastric cancer (GC) incidences in *H. pylori*-treated subjects compared with the expected GC incidence (**a**) in the general population for the whole cohort and (**b**) cumulative incidences of GC in all *H. pylori*–treated subjects ($n = 73{,}237$), after stratification by age at treatment, compared with the expected cumulative incidence in the matched general population. The curves were censored at 13 years. The expected number of GC cases was calculated using data from the Hong Kong Cancer Registry on individuals in the general population with similar age and sex (Adapted from Leung et al. [2])

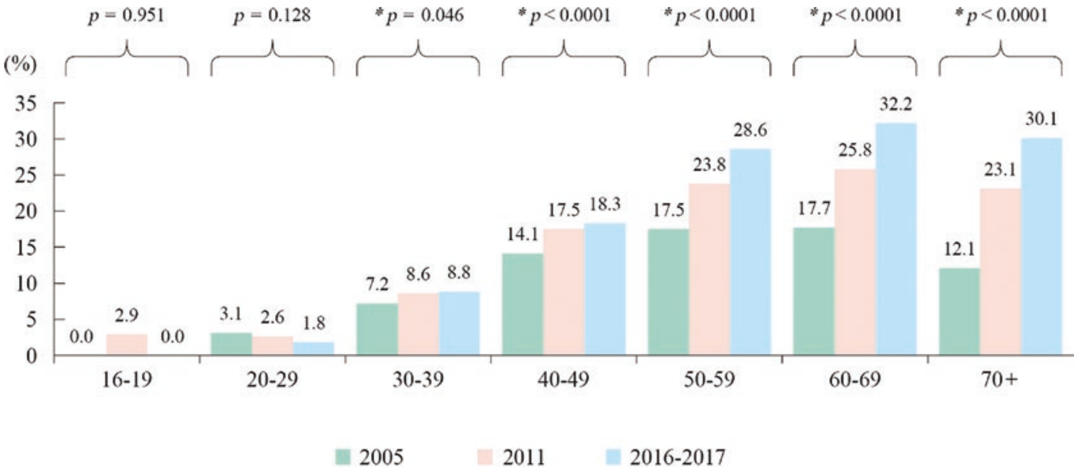

Fig. 56.2 Trends in the rate of *H. pylori* eradication therapy stratified by age group in 2005, 2011, and 2016–2017 (trend p-value). The rate of *H. pylori* eradication therapy increased with increasing age, especially in the 60–69 in 2016–2017 (Adapted from Lim et al. [19])

56.3 Meta-Analyses

In 2009, Fuccio et al. published the first meta-analysis based on RCTs which compared the incidence of GC between the *H. pylori* eradication group and *H. pylori* non-eradication group [20]. In this meta-analysis, a total of seven studies were included. GC developed in 37 (1.1%) of 3388 patients who underwent *H. pylori* eradication and in 56 (1.7%) of 3307 patients who did not treat *H. pylori* infection. When the individual studies in which the follow-up duration was 4–10 years were taken together, the RR of the GC development in the patients who underwent *H. pylori* eradication was 0.65 (95% CI, 0.43–0.98). However, this meta-analysis separately evaluated two studies in which the follow-up duration was different but actually the same data were used. In addition, there was a criticism that when the analysis was performed considering the duplicated two studies as one data, the statistical significance disappeared [21]. Therefore, new meta-analysis was performed for six RCTs which divided *H. pylori*-infected healthy people into the *H. pylori* eradication group and control group and followed them up at least for 2 years [22]. GC developed in 51 (1.6%) of 3294 people who underwent *H. pylori* eradication and in 76 (2.4%) of 3203 people who did not treat *H. pylori* [22]. In the group in which *H. pylori* was successfully eradicated, the RR for the GC development was 0.66 (95% CI, 0.46–0.95) [22]. There was no heterogeneity among included studies [22]. When subgroup analyses were performed according to the presence or absence of associated premalignant lesions, the incidence of GC after *H. pylori* eradication was not different. In addition, when the subjects were followed up during 5–14.7 years, 24 (1.1%) of 2242 in the *H. pylori* eradication group died and 36 (1.6%) of 2233 controls died; the GC-related mortality rate was also significantly different between the two groups (RR in *H. pylori* eradication group 0.67, 95% CI, 0.40–0.11) [22]. However, 192 (7.3%) of 2639 in the *H. pylori* eradication group died and 175 (6.7%) of 2614 controls died; the overall mortality rate was not significantly different (RR 1.09; 95% CI, 0.86–1.38) [22]. Furthermore, the number need to treat (NNT) for the GC development was mean 124. In the subgroup analyses, it was lowest in Chinese male (NNT = 15) and highest in American female (NNT = 245) [22]. The authors claimed that there is evidence that *H. pylori* eradication is effective for GC prevention in asymptomatic healthy people in Asia where the risk of GC is high. However, this meta-analysis also has several limitations. First, only three studies among included six studies have low risk of bias. In addition, because some studies did not have factorial design which can test interaction among the factors, it is difficult to judge whether the prevention of the GC development after *H. pylori* eradication is caused by *H. pylori* eradication itself. Second, all studies except just one study were performed in East Asia. Therefore, it is very difficult to conclude the effect of *H. pylori* eradication in non-Asian countries. Third, the result that the reduced risk of the GC development after *H. pylori* eradication is not related with the presence or absence of precancerous lesions is somewhat difficult to accept. Therefore, it is a thesis to be demonstrated by large RCT in the future. Finally, because the CI of the results was wide, there is a possibility that the combined results may be affected by specific single study among included studies.

In 2020, there was another systematic review and meta-analysis regarding the effect of *H. pylori* eradication on the GC development using 10 RCTs, seven recruited 8323 healthy individuals, and three randomised 1841 patients with gastric neoplasia [23]. In healthy individuals, the eradication therapy reduced the incidence of GC (RR 0.54; 95% CI, 0.40–0.72; NNT = 72), and reduced mortality from GC (RR 0.61; 95% CI, 0.40–0.92; NNT = 135), but did not affect all-cause mortality [23]. These data suggest that 8,743,815 DALYs (95% CI, 5,646,173-11,847,456) would be gained if population screening and treatment is implemented globally [23]. In patients with gastric neoplasia, *H. pylori* eradication therapy also reduced the incidence of future GC (RR 0.49; 95% CI, 0.34–0.70; NNT = 21) [23]. The authors concluded that there was moderate evidence to suggest that *H. pylori* eradication therapy reduced the incidence of GC in healthy individuals and patients with gastric neoplasia in East Asian countries. In addition, they added that there also appeared to be a reduction in GC-related mortality [23].

56.4 Guidelines

In Asia-Pacific area, both consensus guidelines for the prevention of GC and management of *H. pylori* infection state that *H. pylori* eradication is an effective strategy for GC prevention in countries where the incidence of GC is high [24, 25]. However, these guidelines qualify that preventive effect of *H. pylori* eradication for GC is more evident in the cases without precancerous lesions at the time of *H. pylori* eradication and GC can develop even in the patients who received successful *H. pylori* eradication [24]. The guidelines of Korea, Japan, and China which are representative countries where the incidence of GC is high also recommend *H. pylori* eradication for GC prevention [26–28]. In addition, a study to prevent GC in Japan suggests that appropriate timing of *H. pylori* eradication is younger age before the development of AG or IM [29]. This is because that *H. pylori* eradication decreased the prevalence of GC only in patients without premalignant lesions such as AG and IM [9, 10]. Similarly, in a Korean cohort study, the eradication of *H. pylori* could not reduce the GC development in patients with IM [30].

Looking at the guidelines of Western countries in the management of *H. pylori* infection, the Maastricht IV/Florence Consensus Report also recommends *H. pylori* eradication for GC prevention [31]. In the Maastricht VI/Florence Consensus Report in which 41 experts from 29 countries representing global community, the recommendation for the *H. pylori* eradication for GC prevention became much stronger [32]. Another European multicenter guideline about the management of precancerous lesions also recommends *H. pylori* eradication after endoscopic or surgical treatment of gastric neoplasm [33]. Although it is a somewhat dated guideline, the US guideline also considers endoscopic resection of EGC as an established indication for *H. pylori* eradication [34]. In 2017, it was revised, and this recommendation for *H. pylori* eradication after endoscopic treatment of gastric neoplasm became stronger [35].

56.5 The Underlying Mechanisms of Chemopreventive Effect of *H. pylori* Eradication on Gastric Cancer

In spite of an accumulation of data regarding the chemopreventive effect of *H. pylori* eradication, it remains unclear how *H. pylori* eradication prevents GC. From our publication [36], the main mechanisms are summarized as follows: first, it is well known that *H. pylori*-induced AG and IM provoke the development of GC. Regarding the reversibility for AG, it has been nearly established but for IM, it is still controversial. However, evidences are beginning to accumulate even in case of IM. It became evident that a reversible change in IM takes more time after *H. pylori* eradication [36]. Second, when epigenetic studies of *PTPN6, MOS, DCC, CRK,* and *VAV1* were performed in noncancerous gastric specimens in terms of *H. pylori* status, *MOS* was found to be a possible surrogate marker for the GC development but the other genes did not show clear trend [37]. It means that *H. pylori* eradication decreases aberrant DNA methylation in a gene-specific manner [37]. Third, when transforming growth factor-β1 (TGF-β1) and TGF-β1-induced epithelial-mesenchymal transition (EMT) markers were investigated in gastric mucosa, *H. pylori* infection triggered the TGF-β1-induced EMT pathway and caused the emergence of GC stem cells, such as CD44v8–10 [38]. When *H. pylori* was eradicated, these two pathways were inhibited [38, 39]. Actually, *H. pylori* eradication prevented the GC development regardless of tissue type of GC. For instance, an 879 cohort study showed that *H. pylori* eradication significantly decreased the risk of non-cardia GC, especially, diffuse type [36, 40] (Table 56.1).

Table 56.1 The effect of *H. pylori* eradication history on gastric cancer in South Korea (Adapted from Kim et al. [36])

	NCGC (*n* = 879)	Intestinal-type NCGC (*n* = 494)	Diffuse-type NCGC (*n* = 352)
Uninfected	0.30 (0.23 to 0.39)	0.35 (0.25 to 0.49)	0.22 (0.15 to 0.32)
Infected,eradication history (+)	0.35 (0.27 to 0.47)	0.50 (0.36 to 0.70)	0.20 (0.13 to 0.31)
Infected, eradication history (−)	1	1	1

Values are adjusted OR (95% CI). *GC* gastric cancer, *NCGC* non-cardia GC

Recently, there have been increasing evidences that not only *H. pylori* but also non-*H. pylori* microbiota is important in the gastric carcinogenesis. That is, the infected individuals show microbial dysbiosis of gastric microbiota. In recent years, a growing number of studies have focused on gastric microbiota changes following *H. pylori* eradication. A nice systemic review and meta-analysis in nine studies enrolling 546 participants showed that successful *H. pylori* eradication could reverse the gastric microbiota dysbiosis, suggesting a new insight into exploring the role of *H. pylori* and the whole gastric microbiota in gastric carcinogenesis [41]. In detail, alpha diversity indexes increased within 1 month after *H. pylori* eradication; significant differences in gastric microbial community structure between before and after eradication were also seen within 1 month in both the quadruple therapy and triple therapy [41]. The trends of the above-mentioned diversity changes persisted with a follow-up of 6 months [41]. The microbial composition altered significantly after *H. pylori* eradication and the relative abundance of *H. pylori*-related taxa decreased [41]. Accordingly, gastric commonly dominant commensals were enriched [41]. Bioinformatic analyses of microbiota functions showed that bacteria reproduction-related pathways were down-regulated and pathways of gastric acid secretion, etc. were up-regulated [41].

56.6 Conclusions

Because GC develops only in a very small portion of *H. pylori*-infected population and it takes a very long time from *H. pylori* infection to gastric carcinogenesis, it is very difficult to prospectively demonstrate the effect of *H. pylori* eradication on the incidence of GC. Nevertheless, the individual studies and meta-analyses to date mostly suggest that the eradication of *H. pylori* is an effective strategy for the prevention of GC. However, there are still controversies regarding when the optimal timing for *H. pylori* eradication and whether we have to treat *H. pylori* infection in all asymptomatic healthy population to prevent GC. However, recent reports support that *H. pylori* is a useful chemopreventive method not only for young population but also for older individuals. The evidences justify to treat *H. pylori* infection in all asymptomatic healthy population regardless of age. In addition, underlying mechanisms of the chemopreventive effect of *H. pylori* eradication on GC are uncovered in several aspects including the changes of gastric microbiota.

References

1. Conteduca V, Sansonno D, Lauletta G, Russi S, Ingravallo G, Dammacco F. *H. pylori* infection and gastric cancer: state of the art (review). Int J Oncol. 2013;42:5–18.
2. Leung WK, Wong IOL, Cheung KS, Yeung KF, Chan EW, Wong AYS, et al. Effects of *Helicobacter pylori* eradication on the incidence of gastric cancer in older individuals. Gastroenterology. 2018;155:67–75.
3. Fukase K, Kato M, Kikuchi S, et al. Effect of eradication of *Helicobacter pylori* on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. Lancet. 2008;372:392–7.
4. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, et al. Kyoto global consensus report on *Helicobacter pylori* gastritis. Gut. 2015;64:1353–67.
5. Watari J, Chen N, Amenta PS, Fukui H, Oshima T, Tomita T, et al. *Helicobacter pylori* associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. World J Gastroenterol. 2014;20:5461–73.
6. Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infec-

tion and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med. 1991;325:1132–6.

7. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, et al. *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med. 1991;325:1127–31.
8. Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, et al. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. BMJ. 1991;302:1302–5.
9. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, et al. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med. 2001;345:784–9.
10. Wong BC, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, et al. *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. JAMA. 2004;291:187–94.
11. Yan L, Chen Y, Chen F, Tao T, Hu Z, Wang J, et al. Effect of *Helicobacter pylori* eradication on gastric cancer prevention: updated report from a randomized controlled trial with 26.5 years of follow-up. Gastroenterology. 2022;163:154–62.e3.
12. Takenaka R, Okada H, Kato J, Makidono C, Hori S, Kawahara Y, et al. *Helicobacter pylori* eradication reduced the incidence of gastric cancer, especially of the intestinal type. Aliment Pharmacol Ther. 2007;25:805–12.
13. Ogura K, Hirata Y, Yanai A, Shibata W, Ohmae T, Mitsuno Y, et al. The effect of *Helicobacter pylori* eradication on reducing the incidence of gastric cancer. J Clin Gastroenterol. 2008;42:279–83.
14. Lee YC, Chen TH, Chiu HM, Shun CT, Chiang H, Liu TY, et al. The benefit of mass eradication of *Helicobacter pylori* infection: a community-based study of gastric cancer prevention. Gut. 2013;62:676–82.
15. Chiang TH, Chang WJ, Chen SL, Yen AM, Fann JC, Chiu SY, et al. Mass eradication of *Helicobacter pylori* to reduce gastric cancer incidence and mortality: a long-term cohort study on Matsu Islands. Gut. 2021;70:243–50.
16. Choi IJ, Kim CG, Lee JY, Kim YI, Kook MC, Park B, et al. Family history of gastric cancer and *Helicobacter pylori* treatment. N Engl J Med. 2020;382:427–36.
17. Kim N, Park YS, Cho SI, Lee HS, Choe G, Kim IW, et al. Prevalence and risk factors of atrophic gastritis and intestinal metaplasia in a Korean population without significant gastroduodenal disease. Helicobacter. 2008;13:245–55.
18. Joo YE, Park HK, Myung DS, Baik GH, Shin JE, Seo GS, et al. Prevalence and risk factors of atrophic gastritis and intestinal metaplasia: a nationwide multicenter prospective study in Korea. Gut Liver. 2013;7:303–10.
19. Lim SH, Kim N, Kwon JW, Kim SE, Baik GH, Lee JY, et al. Trends in the seroprevalence of *Helicobacter pylori* infection and its putative eradication rate over 18 years in Korea: a cross-sectional nationwide multicenter study. PLoS One. 2018;13:e0204762.
20. Fuccio L, Zagari RM, Eusebi LH, Laterza L, Cennamo V, Ceroni L, et al. Meta-analysis: can *Helicobacter pylori* eradication treatment reduce the risk for gastric cancer? Ann Intern Med. 2009;151:121–8.
21. Ford AC, Moayyedi P. Redundant data in the meta-analysis on *Helicobacter pylori* eradication. Ann Intern Med. 2009;151:513; author reply −4.
22. Ford AC, Forman D, Hunt RH, Yuan Y, Moayyedi P. *Helicobacter pylori* eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials. BMJ. 2014;348:g3174.
23. Ford AC, Yuan Y, Moayyedi P. *Helicobacter pylori* eradication therapy to prevent gastric cancer: systematic review and meta-analysis. Gut. 2020;69:2113–21.
24. Fock KM, Talley N, Moayyedi P, Hunt R, Azuma T, Sugano K, et al. Asia-Pacific consensus guidelines on gastric cancer prevention. J Gastroenterol Hepatol. 2008;23:351–65.
25. Fock KM, Katelaris P, Sugano K, Ang TL, Hunt R, Talley NJ, et al. Second Asia-Pacific consensus guidelines for *Helicobacter pylori* infection. J Gastroenterol Hepatol. 2009;24:1587–600.
26. Jung HK, Kang SJ, Lee YC, Yang HJ, Park SY, Shin CM, et al. Evidence-based guidelines for the treatment of *Helicobacter pylori* infection in Korea 2020. Gut Liver. 2021;15:168–95.
27. Asaka M, Kato M, Takahashi S, Fukuda Y, Sugiyama T, Ota H, et al. Guidelines for the management of *Helicobacter pylori* infection in Japan: 2009 revised edition. Helicobacter. 2010;15:1–20.
28. Chinese Society of Gastroenterology CSGoHp, Liu WZ, Xie Y, Cheng H, Lu NH, Hu FL, et al. Fourth Chinese National consensus report on the management of *Helicobacter pylori* infection. J Dig Dis. 2013;14:211–21.
29. Asaka M. A new approach for elimination of gastric cancer deaths in Japan. Int J Cancer. 2013;132:1272–6.
30. Kim N, Park RY, Cho SI, Lim SH, Lee KH, Lee W, et al. *Helicobacter pylori* infection and development of gastric cancer in Korea: long-term follow-up. J Clin Gastroenterol. 2008;42:448–54.
31. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of *Helicobacter pylori* infection—the Maastricht IV/Florence consensus report. Gut. 2012;61:646–64.
32. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection—the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
33. Dinis-Ribeiro M, Areia M, de Vries AC, Marcos-Pinto R, Monteiro-Soares M, O'Connor A, et al. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European *Helicobacter* Study Group

(EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). Endoscopy. 2012;44:74–94.

34. Chey WD, Wong BC. American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection. Am J Gastroenterol. 2007;102:1808–25.
35. Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG clinical guideline: treatment of *Helicobacter pylori* infection. Am J Gastroenterol. 2017;112:212–39.
36. Kim N. Chemoprevention of gastric cancer by *Helicobacter pylori* eradication and its underlying mechanism. J Gastroenterol Hepatol. 2019;34:1287–95.
37. Shin CM, Kim N, Lee HS, Park JH, Ahn S, Kang GH, et al. Changes in aberrant DNA methylation after *Helicobacter pylori* eradication: a long-term follow-up study. Int J Cancer. 2013;133:2034–42.
38. Choi YJ, Kim N, Chang H, Lee HS, Park SM, Park JH, et al. *Helicobacter pylori*-induced epithelial-mesenchymal transition, a potential role of gastric cancer initiation and an emergence of stem cells. Carcinogenesis. 2015;36:553–63.
39. Choi YJ, Kim N, Lee HS, Park SM, Park JH, Yoon H, et al. Expression of leucine-rich repeat-containing G-protein coupled receptor 5 and CD44: potential implications for gastriccancerstem cell marker. J Cancer Prev. 2016;21:279–87.
40. Oh S, Kim N, Kwon J-W, Shin CM, Choi YJ, Lee DH, et al. Effect of *Helicobacter pylori* eradication and ABO genotype on gastric cancer development. Helicobacter. 2016;21:596–605.
41. Guo Y, Cao XS, Guo GY, Zhou MG, Yu B. Effect of *Helicobacter pylori* eradication on human gastric microbiota: asystematic review and meta-analysis. Front Cell Infect Microbiol. 2022;12:899248.

57

Secondary Prevention of Gastric Cancer After Endoscopic or Surgical Treatment

Nayoung Kim

Abstract

Few reports have shown survival improvements after *Helicobacter pylori* (*H. pylori*) eradication in patients treated with surgery in the early gastric cancer (EGC). Recently, there was a report that it is also beneficial for the better survival in the advanced gastric cancer (AGC) as well. The main reason of this effect is reducing the remote metastasis as well as local recurrence. Several guidelines including the VI/Florence consensus report strongly recommended *H. pylori* eradication in patients with previous gastric neoplasia treated with endoscopic or subtotal gastric resection to reduce the recurrence of gastric cancer (GC) after gastrectomy. However, the underlying mechanism why the *H. pylori* eradication improves the survival of GC patients after surgical treatment is not fully clarified. As immune mechanism determines the survival of advanced cancer patients and immune checkpoint inhibitor was found to be a very powerful tool in the treatment of lung cancer, melanoma and GC one of the underlying mechanisms in the improvement of GC survival by *H. pylori* eradication could be immune mediated.

Keywords

Helicobacter pylori · Eradication · Gastric cancer · Surgery · Endoscopy · Treatment

57.1 Introduction

There have been several reports regarding the survival improvements after *Helicobacter pylori* (*H. pylori*) eradication after surgery or endoscopic treatments in the patients with early gastric cancer (EGC) [1, 2]. Recently, we reported statistically significant benefits of overall and gastric cancer (GC)-specific survival in the *H. pylori*-eradicated group compared to the non-eradicated GC group ($p < 0.001$) [3]. The improvements on the GC-specific survival was more definite in the advanced gastric cancer (AGC) than EGC. Maybe, this is originated from the reason that the survival outcome of EGC is very excellent regardless of *H. pylori* eradication. From this background, *H. pylori* eradication in patients who underwent subtotal gastrectomy or endoscopic treatments for EGC is strongly recommended in several guidelines in the world [4–7] and recent Maastricht VI/Florence consensus report [8]. In this chapter, we focused on the secondary prevention effect of *H. pylori* eradication on the recurrence of GC after surgery or endoscopic treatments.

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57.2 Chemoprevention Effect of *H. pylori* Eradication After Endoscopic Treatments

Several studies have evaluated the effect of *H. pylori* eradication after endoscopic resection of EGC. First, a retrospective Japanese study reported that *H. pylori* eradication in the patients who underwent an endoscopic resection of EGC reduced the incidence of metachronous GC [9]. Thereafter, Fukase et al. reported that the relative risk (RR) for metachronous GC in the *H. pylori* eradication group was 0.35 (95% confidence interval [CI], 0.16–0.78; $p = 0.009$) compared with the control group among subjects who underwent endoscopic submucosal dissection (ESD) for EGC [2] This article had become the key evidence for the recommendation of *H. pylori* eradication in EGC patients in Japan. However, a subsequent Japanese retrospective study reported conflicting results that *H. pylori* eradication could not reduce the incidence of metachronous GC in the patients who underwent ESD for EGC for a long period [10]. Similarly, Korean randomized controlled trial (RCT) in the 901 patients for median 3 years, the incidence of metachronous GC was not significantly different between the *H. pylori* eradication group and control group [11]. However, another Korean RCT reported that *H. pylori* eradication reduces the incidence of metachronous GC in the patients who underwent endoscopic resection of EGC [12]. Recently, the results of meta-analysis of 13 studies including 6237 subjects which evaluated the effect of *H. pylori* eradication on the development of metachronous GC in the subjects who underwent endoscopic resection of EGC were reported [13]. They reported that *H. pylori* eradication in the patients who underwent endoscopic resection of EGC decreased the incidence of metachronous GC approximately by half (odds ratio [OR] 0.42; 95% CI, 0.32–0.56). However, there are some limitations that all included studies were performed in Korea and Japan where the detection rate of EGC is high and ESD is performed very actively. In addition, RCTs were just two [13]. Another limitation is that some studies in this meta-analysis included gastric dysplasia, precancerous lesion of GC, as well as GC into the definition of metachronous neoplasm; thus, the results should be interpreted with caution. Taken together, the results for whether *H. pylori* eradication could prevent metachronous GC and gastric dysplasia after endoscopic resection of EGC are generally accepted nowadays. Recently, Korean government included gastric dysplasia as well as EGC as an indication of *H. pylori* eradication and medical insurance covers *H. pylori* eradication treatments for these diseases.

57.3 Chemoprevention Effect of *H. pylori* Eradication After Surgery

Well-designed studies which evaluated the effect of *H. pylori* eradication in the patients who underwent surgery for GC are rare. These studies are rather difficult because the biochemical and microbiological composition of gastric juice after gastrectomy dramatically change as along with the change of anatomical structures [14]. In addition, there might be a possibility that bile reflux could act as carcinogen in these patients [15]. There was a report from Korean which randomly allocated 190 patients who underwent subtotal gastrectomy for GC into the *H. pylori* eradication group and placebo-administrated group and followed them up for 36 months [16]. The incidence of metachronous GC was not different between the two groups and according to the final *H. pylori* infection status of the patient [16]. However, *H. pylori* eradication improved premalignant conditions after gastrectomy [17, 18].

Recently, our team reported benefits of *H. pylori* eradication in the 1031 patients who received surgical treatment for GC [3]. A total of 1031 patients were enrolled, and 564 (54.7%) patients were not treated for *H. pylori*. Among 467 patients, 451 (43.7%) patients were successfully eradicated, and the eradication failed in 16 (1.6%) patients [3] (Fig. 57.1). The overall characteristics of the enrolled patients and inter-group differences allocated by *H. pylori* eradication are shown in Table 57.1 [3]. The median age was 59 years (interquartile range [IQR], 49–68 years),

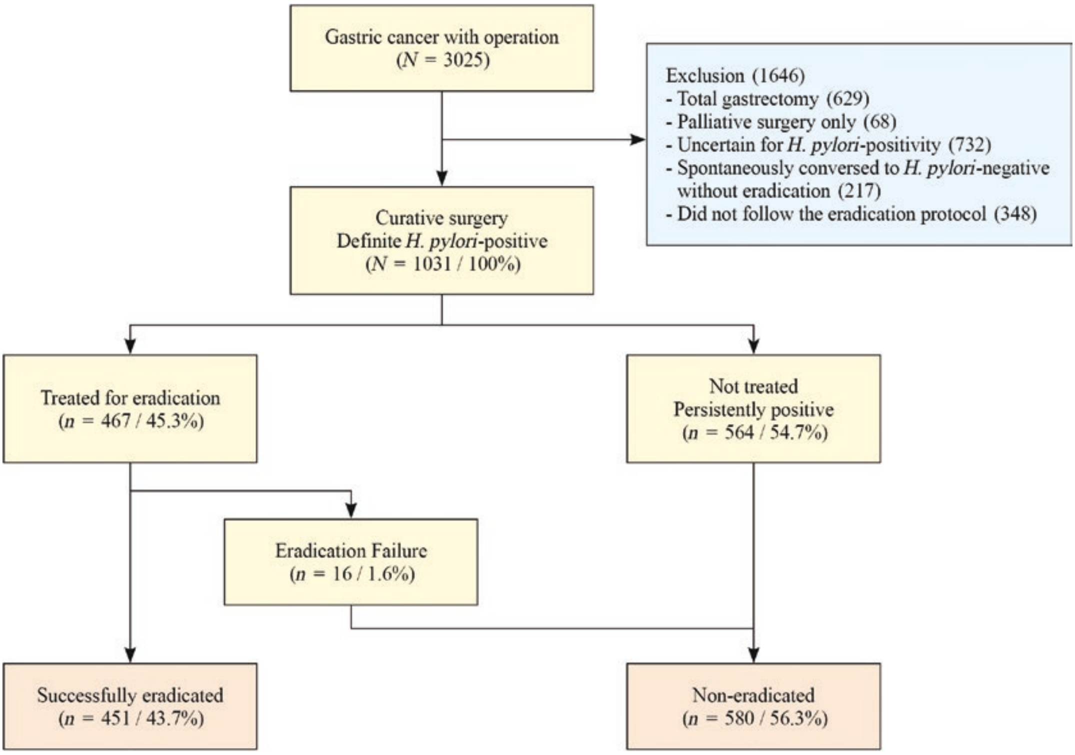
Gastric cancer with operation
(N = 3025)
Exclusion (1646)
- Total gastrectomy (629)
- Palliative surgery only (68)
- Uncertain for *H. pylori*-positivity (732)
- Spontaneously conversed to *H. pylori*-negative without eradication (217)
- Did not follow the eradication protocol (348)
Curative surgery
Definite *H. pylori*-positive
(N = 1031 / 100%)
Treated for eradication
(n = 467 / 45.3%)
Not treated
Persistently positive
(n = 564 / 54.7%)
Eradication Failure
(n = 16 / 1.6%)
Successfully eradicated
(n = 451 / 43.7%)
Non-eradicated
(n = 580 / 56.3%)

Fig. 57.1 Study algorithm for the inclusion and classification of 1031 patients who received surgical treatment for gastric cancer depending on *H. pylori* eradication (Adapted from Choi et al. [3])

and the median follow-up period was 67 months (IQR, 69–124 months, maximum 179 months; 306 patients had longer than 10-year follow-up period) EGC patients were 65.8% and AGC patients were 34.2% of overall, which is consistent with the previous surveys of Korea. Patients in the *H. pylori* eradicated group were statistically younger, had an earlier stage cancer, underwent Billroth I reconstruction compared to the patients in the non-eradicated group [3] (Table 57.1). However, there was no difference in terms of adjuvant chemotherapy (p = 0.986) (Table 57.1).

Totally, 66 patients out of 1031 (6.4%) were found to be recurred, 8 patients in EGC group (8 out of 678, 1.2%) and 58 patients in AGC group (58 out of 353, 16.4%) [3]. The incidence of cancer recurrence was higher in the non-eradicated group (56 out of 580, 9.6%) compared to the treatment group (10 out of 451, 2.2%) [3] (Table 57.2). The recurrence sites are as following: in the eradicated group (10 cases), recurred sites were peritoneal seeding (including cases which were difficult to verify the exact location of the recurrence such as malignant ascites, multiple abdominal and pelvic cavity lymph node enlargements, or adhesive ileus, 60%), anastomosis site recurrence (30%), bone metastases (10%) [3]. In the non-eradicated group, recurred sites were peritoneal seeding (60%), hepatobiliary metastases (25%), anastomosis site recurrence (7%), lung or thorax lymph node enlargement (4%), and brain or leptomeningeal metastases (4%). In both groups, peritoneal seeding was more than a half since most recurred cancer cases were revealed as an advanced cancer stage. In the eradicated group, anastomosis site recurrence was the second, while hepatobiliary metastases were the second in the non-eradicated group. The number of recurred cases were too small to find statistical significance.

Among several variables, cancer stage (cancer stage II, adjusted hazard ratio [aHR] = 7.08; $p < 0.001$; cancer stage III or IV, aHR = 19.64; $p < 0.001$) and *H. pylori* positivity (aHR = 2.70;

Table 57.1 Multivariate analysis regarding clinical and pathological factors associated with gastric cancer-specific mortality (Adapted from Choi et al. [3])

		Univariable analysis		Multivariable analysis	
Factor	*n*	cHR (95% CI)	*p*-value	aHR (95% CI)	*p*-value
Sex					
F	364	Ref			
M	667	0.91 (0.58–1.44)	0.696		
Age					
<60	535	Ref		Ref	
≥60	496	1.60 (1.02–2.50)	**0.040**	1.52 (0.96–2.42)	0.078
Operation method					
Billroth-I	641	Ref		Ref	
Billroth-II	316	1.85 (1.18–2.90)	**0.007**	1.23 (0.78–1.94)	0.372
Proximal gastrectomy or wedge resection	74	0.45 (0.11–1.88)	0.276	0.92 (0.22–3.82)	0.903
Family Hx.					
No	854	Ref			
Yes	177	0.86 (0.46–1.59)	0.626		
Smoking Hx.					
No	570	Ref			
Yes	461	1.31 (0.84–2.03)	0.233		
Alcohol Hx.					
No	534	Ref			
Yes	497	0.99 (0.64–1.54)	0.964		
Atrophic gastritis					
No	743	Ref			
Yes	288	0.99 (0.60–1.61)	0.958		
Intestinal metaplasia					
No	592	Ref			
Yes	439	1.00 (0.64–1.55)	0.981		
Histologic type					
Intestinal	542	Ref		Ref	
Diffuse	436	2.17 (1.36–3.46)	**0.001**	1.57 (0.96–2.55)	0.072
Mixed	53	1.08 (0.33–3.57)	0.895	0.91 (0.27–3.03)	0.879

(continued)

Table 57.1 (continued)

		Univariable analysis		Multivariable analysis	
Factor	*n*	cHR (95% CI)	*p*-value	aHR (95% CI)	*p*-value
Final cancer stage after surgery					
I	785	Ref		Ref	
II	156	12.45 (6.33–4.49)	**<0.001**	9.33 (4.70–18.54)	**<0.001**
III or more	90	38.69 (20.23–4.03)	**<0.001**	26.17 (13.37–51.21)	**<0.001**
Adjuvant chemotherapy					
No	825	Ref			
Yes	206	0.72 (0.39–1.33)	0.292		
HP eradication					
Successfully eradicated	451	Ref		Ref	
Eradication failure or not treated	580	6.66 (3.33–13.33)	**<0.001**	3.41 (1.68–6.94)	**0.001**

Cox proportional hazards model was used for uni- and multivariable analyses; $p < 0.2$ were used for multivariable analyses

HP Helicobacter pylor, cHR crude hazard ratio, *CI* confidence interval, *aHR* adjusted hazard ratio

Bold style indicates statistical significance

Table 57.2 Multivariate analysis regarding clinical and pathological factors associated with gastric cancer recurrence (Adapted from Choi et al. [3])

		Univariable analysis		Multivariable analysis	
Factor	***n***	cHR (95% CI)	*p*-value	aHR (95% CI)	*p*-value
Sex					
F	364	Ref			
M	667	0.87 (0.53–1.43)	0.579		
Age					
<60	535	Ref			
≥60	496	1.14 (0.71–1.85)	0.584		
Operation method					
Billroth-I	641	Ref		Ref	
Billroth-II	316	1.95 (1.19–3.20)	**0.008**	1.35 (0.82–2.24)	0.238
Proximal gastrectomy or wedge resection	74	0.58 (0.14–2.40)	0.450	1.15 (0.27–4.88)	0.845
Family Hx.					
No	854	Ref			
Yes	177	0.97 (0.51–1.85)	0.918		

(continued)

Table 57.2 (continued)

Factor	***n***	Univariable analysis		Multivariable analysis	
		cHR (95% CI)	*p*-value	aHR (95% CI)	*p*-value
Smoking Hx.					
No	570	Ref			
Yes	461	1.37 (0.84–2.22)	0.204		
Alcohol Hx.					
No	534	Ref			
Yes	497	0.94 (0.58–1.53)	0.803		
Atrophic gastritis					
No	743	Ref			
Yes	288	0.82 (0.47–1.44)	0.493		
Intestinal metaplasia					
No	592	Ref			
Yes	439	0.86 (0.53–1.41)	0.558		
Histologic type					
Intestinal	542	Ref		Ref	
Diffuse	436	2.39 (1.43–4.00)	**0.001**	1.61 (0.95–2.72)	0.076
Mixed	53	0.90 (0.21–3.82)	0.885	0.78 (0.18–3.36)	0.742
Final cancer stage after surgery					
I	785	Ref		Ref	
II	156	9.08 (4.57–18.03)	**<0.001**	7.08 (3.52–14.23)	**<0.001**
III or more	90	28.49 (14.88–54.53)	**<0.001**	19.64 (9.99–38.63)	**<0.001**
Adjuvant chemotherapy					
No	825	Ref		Ref	
Yes	206	0.55 (0.26–1.14)	0.109	0.57 (0.27–1.19)	0.131
HP eradication					
Successfully eradicated	451	Ref		Ref	
Eradication failure or not treated	580	5.04 (2.57–9.90)	**< 0.001**	2.70 (1.35–5.38)	**0.005**

Cox proportional hazards model was used for uni- and multivariable analyses; $p < 0.2$ were used for multivariable analyses

HP Helicobacter pylori, cHR crude hazard ratio, *CI* confidence interval, *aHR* adjusted hazard ratio

Bold style indicates statistical significance *p* = 0.005) were independent risk factors for cancer recurrence [3] (Table 57.2). These results suggest that *H. pylori* eradication be conducted more intensively in patients who are surgically treated for GC, regardless of cancer stage. These analyses were performed from the surgical and medical cohort which were built from 2003 but *H. pylori* eradication was not performed randomly. Thus, further randomized clinical trial is necessary to verify this promising result.

In the surgical cohort, the risk factors for metachronous GC development including age, sex, smoking, alcohol, *H. pylori* status, family history of GC, histological types, and surgical method were analysed [19] (Table 57.3). In the remnant stomach metachronous GC occurred in the 35 patients among 3045 patients (1.1%) in the remaining stomach: 23 in the distal gastrectomy (DG) group (18 with Billroth-I anastomosis, 5 with Billroth-II anastomosis), 7 in the proximal gastrectomy (PG) group, and 5 in the pylorus-preserving gastrectomy (PPG) group [19] (Fig. 57.2). Cox univariate and multivariate analyses have identified that age ≥ 60 ($p = 0.005$) and surgical methods used (PG or PPG, $p < 0.001$) were related risk factors for meta-

Table 57.3 Baseline characteristics in the patients with gastrectomy for gastric cancer depending on metachronous gastric cancer (Adapted from Choi et al. [19])

Variable	Patients with metachronous gastric cancer (*n* = 35)	Patients without metachronous gastric cancer (*n* = 3010)	*p*-value
Sex			
Female	7	998	0.100
Male	28	2012	
Mean age (year ± SD)	66.09 ± 7.58	60.10 ± 12.38	**<0.001**
Family history of GC			
No	29	2514	0.918
Yes	6	496	
Smoking status			
No	16	1668	0.253
Yes	19	1342	
Alcohol drinking			
No	19	1648	0.960
Yes	16	1362	
Gastric atrophy			
Absent	21	2293	**0.026**
Present	14	717	
Intestinal Metaplasia			
Absent	13	1879	**0.002**
Present	22	1131	
Histologic type (Lauren's classification)			
Intestinal	28	1650	**0.008**
Diffuse	5	1190	
Mixed or undifferentiated	2	170	
Helicobacter pylori status			
Eradicated	10	771	0.368
Negative	20	1408	
Positive (not treated)	5	831	

(continued)

Table 57.3 (continued)

Variable	Patients with metachronous gastric cancer ($n = 35$)	Patients without metachronous gastric cancer ($n = 3010$)	*p*-value
Surgical method for initial GC			
Billroth-I	18	1839	**< 0.001**
Billroth-II	5	962	
Proximal gastrectomy	7	139	
Pylorus-preserving gastrectomy	5	93	

Bold style indicates statistical significance ($p < 0.05$)
SD standard deviation, *GC* gastric cancer

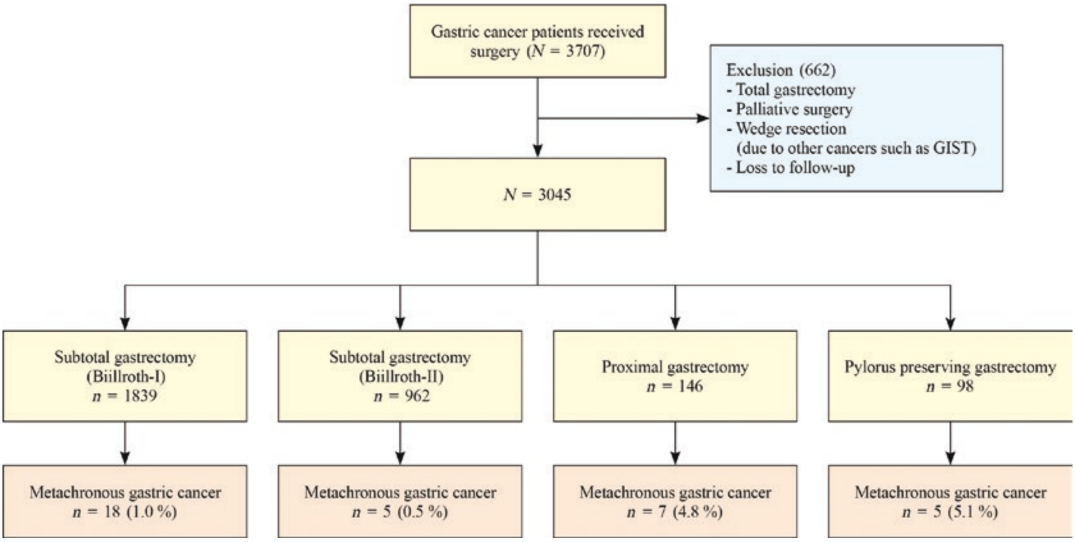
Gastric cancer patients received surgery (N = 3707)
Exclusion (662)
- Total gastrectomy
- Palliative surgery
- Wedge resection
(due to other cancers such as GIST)
- Loss to follow-up
N = 3045
Subtotal gastrectomy (Biillroth-I) n = 1839
Subtotal gastrectomy (Biillroth-II) n = 962
Proximal gastrectomy n = 146
Pylorus preserving gastrectomy n = 98
Metachronous gastric cancer n = 18 (1.0 %)
Metachronous gastric cancer n = 5 (0.5 %)
Metachronous gastric cancer n = 7 (4.8 %)
Metachronous gastric cancer n = 5 (5.1 %)

Fig. 57.2 Study flow chart in the remnant stomach of 3045 patients. 35 patients metachronous gastric cancer occurred (Adapted from Choi et al. [19])

chronous GC, while male sex and intestinal type histology were potential risk factors [19] (Table 57.4). However, there was no definite statistical significance depending on *H. pylori* eradication [19] (Table 57.4). Instead, metachronous GC was shown to be related to older age and surgical methods used (PG or PPG) [19]. These results suggest that regular, careful follow-up with endoscopy should be performed in the case of gastric partial resection, especially in patients with male sex and intestinal type histology as well as age ≥ 60 and PG/PPG surgical methods [19].

Table 57.4 Risk factors for metachronous gastric cancer in the remnant stomach (Adapted from Choi et al. [19])

	Univariate analyses		Multivariate analyses	
Variable	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Sex				
Female	Ref	**0.048**	Ref	0.266
Male	2.30 (1.01–5.29)		1.62 (0.69–3.81)	
Age				
<60	Ref	**0.002**	Ref	**0.018**
≥60	3.74 (1.63–8.56)		2.78 (1.19–6.49)	
Family history of GC				
No	Ref	0.975		
Yes	0.99 (0.41–2.38)			
Smoking status				
No	Ref	0.156		
Yes	1.62 (0.83–3.15)			
Alcohol drinking				
No	Ref	0.992		
Yes	1.00 (0.51–1.94)			
Gastric atrophy				
Absent	Ref	0.129		
Present	1.69 (0.86–3.33)			
Intestinal Metaplasia				
Absent	Ref	**0.011**	Ref	0.133
Present	2.43 (1.23–4.84)		1.71 (0.85–3.46)	
Histologic type (Lauren's classification)				
Intestinal	Ref	**0.014**	Ref	0.189
Diffuse	0.24 (0.09–0.63)		0.40 (0.15–1.08)	
Mixed or undifferentiated	0.66 (0.16–2.80)		0.77 (0.18–3.35)	
Helicobacter pylori				
Eradicated	Ref	0.087	Ref	0.429
Negative	2.18 (1.00–4.75)		1.69 (0.75–3.82)	
Positive (not treated)	1.02 (0.34–3.02)		1.19 (0.39–3.60)	
Surgical method for initial GC				
Billroth-I	Ref	**<0.001**	Ref	**<0.001**
Billroth-II	0.65 (0.29–2.16)		0.84 (0.31–2.29)	
Proximal gastrectomy	7.60 (3.10–18.61)		6.71 (2.66–16.94)	
Pylorus-preserving gastrectomy	10.27 (3.66–28.81)		8.93 (3.12–25.54)	

Bold style indicates statistical significance ($p < 0.05$)
OR odds ratio, *CI* crude interval, *GC* gastric cancer

57.4 Survival Benefits of *H. pylori* Eradication After Surgery

Several studies in the patients who underwent surgical resection for GC did not show definite favorable results to date regarding improving survival. Recently, our study suggested that *H. pylori* eradication after subtotal gastrectomy brought significant benefit of overall- or GC-specific survival in a large cohort and long-term follow-up study (over 300 patients with follow-up longer than 10 years) [3] (Fig. 57.3). In addition, statistically significant survival benefits were observed in sub-group analysis according to cancer type such as EGC and AGC, before and after propensity score matching (PSM). PSM is used to match patients with a similar distribution of confounders so that the difference in outcomes gives an unbiased estimate of treatment effect, thereby increasing between-group comparability [20, 21]; therefore, it is increasingly being used in observational studies recently, especially when RCT is difficult to perform [22, 23]. Furthermore, *H. pylori* positivity as well as final cancer stage were independent risk factors for GC-specific death in Cox proportional hazards in the univariate and multivariate analyses [3] (Fig. 57.3). Moreover, *H. pylori* positivity and final cancer stage were independent risk factors for cancer recurrence [3] (Fig. 57.3). Actually, this was the first report to date showing the survival gain of *H. pylori* eradication after subtotal gastrectomy not only in EGC (Figs. 57.4a and c) but also in AGC [3] (Figs. 57.4b and d). There might be several reasons for this benefit. First, *H. pylori* plays an important role in the recurrence of GC, so *H. pylori* eradication reduced the recurrence of GC in patients treated with endoscopic resection of GC, naturally leading to improved survival [1, 2]. Second, *H. pylori* plays an important role in the extraintestinal diseases. That is, several recent studies have reported on the systemic effects of *H. pylori*, such as hypertension and hyperlipidemia. This is supported by a report describing survival improvement after *H. pylori* eradication

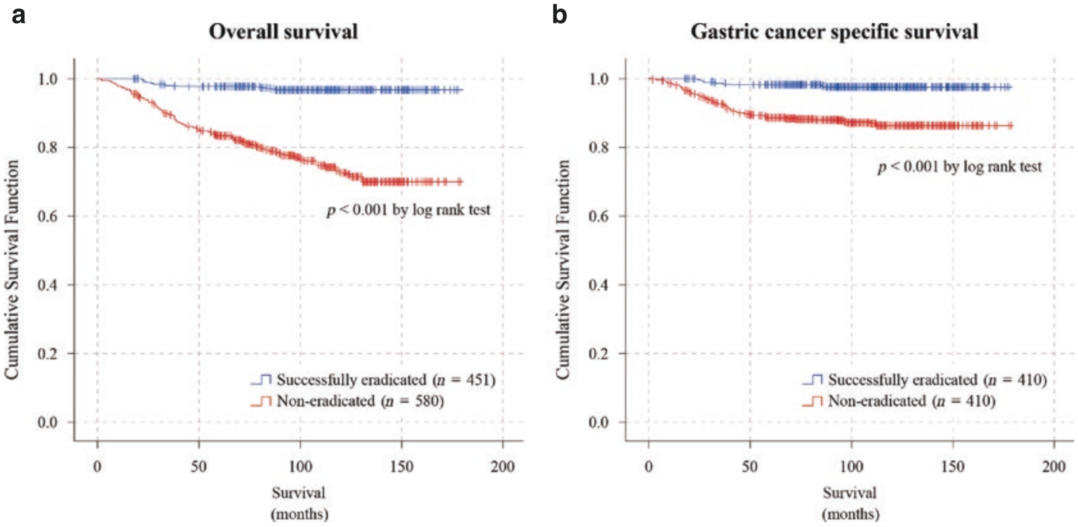

Fig. 57.3 Comparisons of overall and gastric cancer-specific survival depending on *H. pylori* eradication. (**a**) The benefits of overall survival and (**b**) gastric cancer-specific survival were observed in the successfully eradicated group compared to non-eradicated group, with statistical significance ($p < 0.001$). Cumulative survival was calculated using Kaplan-Meier estimates; the *p*-values were calculated using the log-rank test (Adapted from Choi et al. [3])

in patients with hypertension [24] and an independent role of *H. pylori* in the pathogenesis of metabolic syndrome [25]. There are other reports about the contribution of *H. pylori* to arterial stiffness [26] and non-alcoholic fatty liver disease [27]. The decrease in these extraintestinal effects of *H. pylori* after eradication might contribute to survival improvements [28]. Third, although we matched the baseline clinical characteristics of both groups using PSM, it is still possible that there were differences between the two groups in key details. *H. pylori* tests and eradication treatments may have been actively conducted in patients who were in relatively good physical condition, who were thought to be able to withstand the side effects of antibiotic treatment such as gastrointestinal disorders, and who had good systemic conditions and an active willingness to treat. Fourth, this promising result might be originated from high proportion of stage I (76.3%) in this GC cohort and because outcome of GC in Koreans was highest in the world [29]. However, when we performed sub-analysis in the EGC and AGC separately to evaluate this point, the benefit of *H. pylori* eradication was much higher in the AGC (Figs. 57.4b and 4d) than that of EGC (Figs. 57.4a and 4c). As the 5-year survival rate of EGC patients is over 95% in Korea, it could be interpreted that the benefit of *H. pylori* eradication in AGC patients contributed a differ-

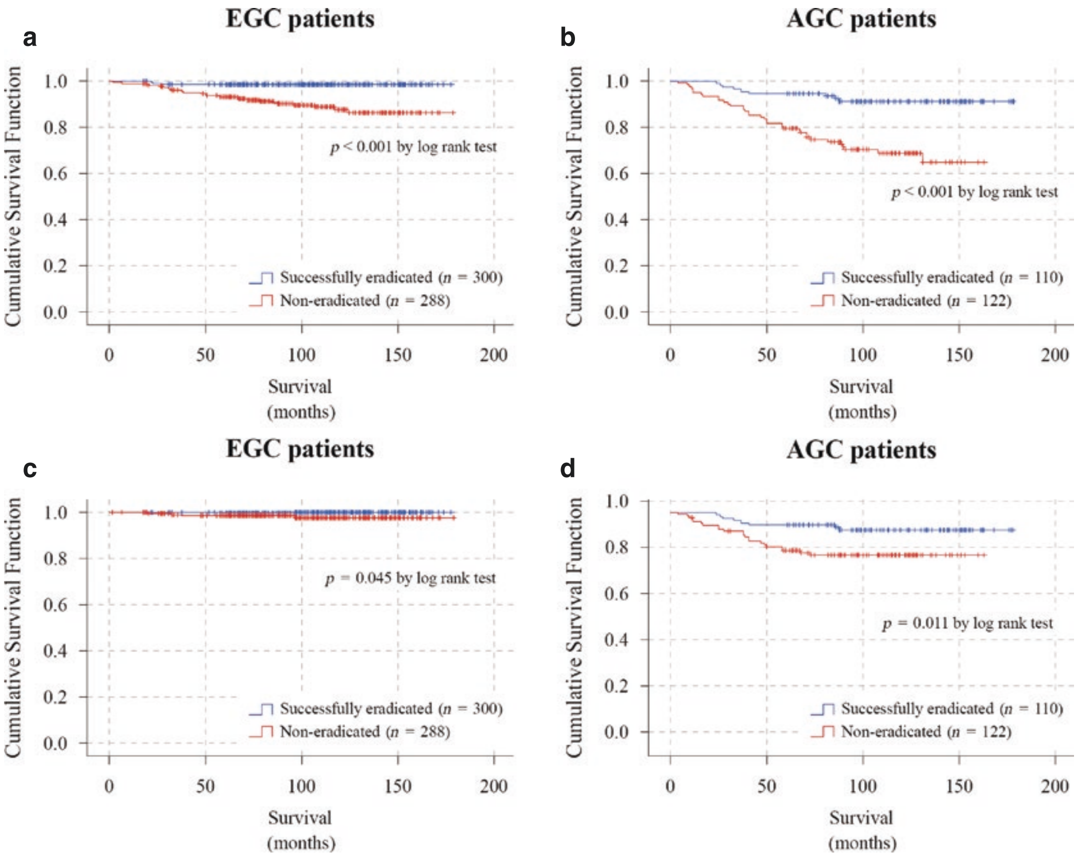

Fig. 57.4 Comparisons of overall and gastric cancer-specific survival depending on *H. pylori* eradication and cancer type after propensity score matching. Overall survival in early gastric cancer (EGC) (**a**) and advanced gastric cancer (AGC) (**b**) patients, gastric cancer-specific survival in EGC (**c**) and AGC (**d**) patients. The benefits of gastric cancer-specific survival were observed in the successfully eradicated group compared to non-eradicated group in all cases, with statistical significance. Cumulative survival was calculated using Kaplan-Meier estimates; the *p*-values were calculated using the log-rank test (Adapted from Choi et al. [3])

ence in whole GC patients [3]. Finally, the underlying mechanism could be immune mechanism although the definite evidence is not clarified, yet. However, as it is well known that the immune mechanism determines the survival of advance cancer patients and immune checkpoint inhibitor was found to be a very powerful tool in the treatment of lung cancer, melanoma and GC, the hypothesis looks like possible. Especially, the cytotoxins of *H. pylori* such as VacA and CagA are known to intervene the immune pathogenesis of GC that if the carcinogenic *H. pylori* is eradicated, then immune mechanism could be activated to deal with the tumor microenvironment. However, deep comprehensive research is needed to verify this presumption in the future.

57.5 Conclusions

There were several studies regarding the secondary chemoprevention effect of *H. pylori* eradication after endoscopic treatments. Initially, the effect was not definite but nowadays, meta-analysis showed the benefits of *H. pylori* eradication. In addition, a survival improvement after *H. pylori* eradication in patients treated with surgery has been reported from a larger scale and with long-term follow-up data [3]. These benefits were related with less GC metastasis after *H. pylori* eradication in the AGC patients. These results suggest that the indication of future *H. pylori* treatment guideline needs to be extended and that intensive screening and treatment for *H. pylori* should be conducted in patients undergoing surgical treatment of GC regardless of the cancer stage. However, further clinical trials are needed to evaluate the effect of *H. pylori* eradication after surgery. In addition, comprehensive research is needed to investigate the underlying mechanisms of survival benefits by *H. pylori* eradication after surgery in the future.

References

1. Choi IJ, Kook MC, Kim YI, Cho SJ, Lee JY, Kim CG, et al. *Helicobacter pylori* therapy for the prevention of metachronous gastric cancer. N Engl J Med. 2018;378:1085–9.
2. Fukase K, Kato M, Kikuchi S, Inoue K, Uemura N, Okamoto S, et al. Effect of eradication of *Helicobacter pylori* on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. Lancet. 2008;372:392–7.
3. Choi Y, Kim N, Yun CY, Choi YJ, Yoon H, Shin CM, et al. Effect of *Helicobacter pylori* eradication after subtotal gastrectomy on the survival rate of patients with gastric cancer: follow-up for up to 15 years. Gastric Cancer. 2020;23:1051–63.
4. Fock KM, Talley N, Moayyedi P, Hunt R, Azuma T, Sugano K, et al. Asia-Pacific consensus guidelines on gastric cancer prevention. J Gastroenterol Hepatol. 2008;23:351–65.
5. Shiota S, Murakami K, Fujioka T, Yamaoka Y. Population-based strategies for *Helicobacter pylori*-associated disease management: a Japanese perspective. Expert Rev Gastroenterol Hepatol. 2010;4:149–56.
6. Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG clinical guideline: treatment of *Helicobacter pylori* infection. Am J Gastroenterol. 2017;112:212–39.
7. Chey WD, Wong BC. American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection. Am J Gastroenterol. 2007;102:1808–25.
8. Malfertheiner P, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, et al. Management of *Helicobacter pylori* infection—the Maastricht VI/Florence consensus report. Gut. 2022;71:1724–62.
9. Uemura N, Mukai T, Okamoto S, Yamaguchi S, Mashiba H, Taniyama K, et al. Effect of *Helicobacter pylori* eradication on subsequent development of cancer after endoscopic resection of early gastric cancer. Cancer Epidemiol Biomark Prev. 1997;6:639–42.
10. Maehata Y, Nakamura S, Fujisawa K, Esaki M, Moriyama T, Asano K, et al. Long-term effect of *Helicobacter pylori* eradication on the development of metachronous gastric cancer after endoscopic resection of early gastric cancer. Gastrointest Endosc. 2012;75:39–46.
11. Choi IJ, Kim SG, Yoon H, Im JP, Kim JS, Kim WH, et al. Eradication of *Helicobacter pylori* after endoscopic resection of gastric tumors does not reduce incidence of metachronous gastric carcinoma. Clin Gastroenterol Hepatol. 2014;12:793–800.

12. Bae SE, Jung HY, Kang J, Park YS, Baek S, Jung JH, et al. Effect of *Helicobacter pylori* eradication on metachronous recurrence after endoscopic resection of gastric neoplasm. Am J Gastroenterol. 2014;109:60–7.
13. Yoon SB, Park JM, Lim CH, Cho YK, Choi MG. Effect of *Helicobacter pylori* eradication on metachronous gastric cancer after endoscopic resection of gastric tumors: a meta-analysis. Helicobacter. 2014;19:243–8.
14. Lin YS, Chen MJ, Shih SC, Bair MJ, Fang CJ, Wang HY. Management of *Helicobacter pylori* infection after gastric surgery. World J Gastroenterol. 2014;20:5274–82.
15. Lu B, Li M. *Helicobacter pylori* eradication for preventing gastric cancer. World J Gastroenterol. 2014;20:5660–5.
16. Cho SJ, Choi IJ, Kook MC, Yoon H, Park S, Kim CG, et al. Randomised clinical trial: the effects of *Helicobacter pylori* eradication on glandular atrophy and intestinal metaplasia after subtotal gastrectomy for gastric cancer. Aliment Pharmacol Ther. 2013;38:477–89.
17. Koulis A, Buckle A, Boussioutas A. Premalignant lesions and gastric cancer: current understanding. World J Gastrointest Oncol. 2019;11:665–78.
18. Hwang JJ, Lee DH, Kang KK, Lee AR, Yoon H, Shin CM, et al. Eradication rate and histological changes after *Helicobacter pylori* eradication treatment in gastric cancer patients following subtotal gastrectomy. World J Gastroenterol. 2015;21:3936–43.
19. Choi Y, Kim N, Yoon H, Shin CM, Park YS, Lee DH, et al. The incidence and risk factors for metachronous gastric cancer in the remnant stomach after gastric cancer surgery. Gut Liver. 2022;16:366–74.
20. Benedetto U, Head SJ, Angelini GD, Blackstone EH. Statistical primer: propensity score matching and its alternatives. Eur J Cardiothorac Surg. 2018;53:1112–7.
21. Baek S, Park SH, Won E, Park YR, Kim HJ. Propensity score matching: a conceptual review for radiology researchers. Korean J Radiol. 2015;16:286–96.
22. Lee SW, Kwon JH, Lee HL, Yoo SH, Nam HC, Sung PS, et al. Comparison of tenofovir and entecavir on the risk of hepatocellular carcinoma and mortality in treatment-naïve patients with chronic hepatitis B in Korea: a large-scale, propensity score analysis. Gut. 2020;69:1301–8.
23. Guo CG, Cheung KS, Zhang F, Chan EW, Chen L, Wong IC, Leung WK. Incidences, temporal trends and risks of hospitalisation for gastrointestinal bleeding in new or chronic low-dose aspirin users after treatment for *Helicobacter pylori*: a territory-wide cohort study. Gut. 2020;69:445–52.
24. Kim YI, Kim YA, Lee JW, Kim HJ, Kim SH, Kim SG, et al. Effect of *Helicobacter pylori* treatment on long-term mortality in patients with hypertension. Gut Liver. 2020;14:47–56.
25. Lim SH, Kim N, Kwon JW, Kim SE, Baik GH, Lee JY, et al. Positive association between *Helicobacter pylori* infection and metabolic syndrome in a Korean population: a multicenter nationwide study. Dig Dis Sci. 2019;64:2219–30.
26. Choi JM, Lim SH, Han YM, Lee H, Seo JY, Park HE, et al. Association between *Helicobacter pylori* infection and arterial stifness: results from a large cross-sectional study. PLoS One. 2019;14:e0221643.
27. Chen C, Zhang C, Wang X, Zhang F, Zhang Z, Ma P, et al. *Helicobacter pylori* infection may increase the severity of nonalcoholic fatty liver disease via promoting liver function damage, glycometabolism, lipid metabolism, infammatory reaction and metabolic syndrome. Eur J Gastroenterol Hepatol. 2020;32:857–66.
28. Kim SB, Kim N, Park J, Hwang IC, Lim SH, Song DH, et al. Preventive effect of *Helicobacter pylori* eradication on the coronary heart diseases depending on age and sex with a median follow-up of 51 months. Helicobacter. 2023;28:e12969.
29. Information Committee of Korean Gastric Cancer Association. Korean gastric cancer association nationwide survey on gastric cancer in 2014. J Gastric Cancer. 2016;16:131–40.

58

Extraintestinal Manifestations Depending on Sex/Gender and Age: Metabolic Parameters and Glycosylated Hemoglobin A1c

Nayoung Kim

Abstract

Helicobacter pylori (*H. pylori*) has been reported to be associated with many extraintestinal manifestations such as endocrine, cardiovascular, hematologic, neurologic, and autoimmune diseases. Thus, these *H. pylori*-induced extraintestinal manifestations are expected to be reversed by *H. pylori* eradication. However, the effects of *H. pylori* eradication on the extraintestinal diseases such as metabolic syndromes or heart diseases remain controversial. These might be related with that the extraintestinal manifestations develop due to other causes in addition to *H. pylori* infection including its secreted protein CagA (cytotoxicity associated gene A). Furthermore, if these *H. pylori* infection-related metabolic syndrome, type 2 diabetes mellitus (T2DM)/prediabetes (preDM), and heart disease differently manifest depending on age and sex/gender, then the effects of *H. pylori* eradication could be different. For instance, high-density lipoprotein (HDL) increased in females after *H. pylori* eradication but not in males. Body mass index increased in males but not females at the time point of 1 year after *H. pylori* eradication. Furthermore, *H. pylori* eradication decreased HbA1c levels in patients with T2DM or preDM over a long-term follow-up period, especially in males and subjects <65 years of age. Taken together sex hormone levels such as estrogen which is related with age might affect *H. pylori*-induced extraintestinal manifestations and its effect of *H. pylori* eradication.

Keywords

Helicobacter pylori · Eradication · Metabolic syndromes · Type 2 diabetes mellitus Prediabetes · Extraintestinal manifestations Sex · Gender · Age

58.1 Introduction

Helicobacter pylori (*H. pylori*), a Gram-negative bacterium that infects the human gastric epithelium, is one of the most common chronic bacterial infections, with a prevalence of more than 50% worldwide [1–3]. *H. pylori* is a type I carcinogen that causes chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma [3]. In addition to gastrointestinal (GI) diseases, *H. pylori* has been reported to be associated with many extraintestinal manifestations such as endocrine [4, 5], cardiovascular [6], hemato-

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logic, neurologic [7], allergic diseases including asthma [8] and autoimmune diseases [9, 10]. Metabolic syndrome, which is characterized by clusters of metabolic abnormalities, has been highlighted as a risk factor for various GI diseases such as fatty liver and hepatoma [11, 12] as well as cardiovascular diseases. Thus, metabolic syndrome places a substantial burden on public health. In practice, the prevalence of metabolic syndrome is increasing steadily worldwide [12–14], and South Korea is no exception. That is, the prevalence of metabolic syndrome in South Korea increased from 19.6–24.9% in 1998 [15, 16] to 28.9–30.5% in 2013 [17, 18]. In addition, the prevalence of metabolic syndrome was increasing at an annual rate of 0.7% from 1998 to 2007 in a South Korean population [15] and then 0.4% from 2009 to 2013 [18]. Interestingly, the prevalence of metabolic syndrome as well as that of *H. pylori* infection, increase with age, and both disorders are affected by socioeconomic factors [15]. If *H. pylori* induces these extraintestinal manifestations naturally, then *H. pylori* eradication should reverse these disorders. However, the effect of *H. pylori* eradication on most of extraintestinal manifestations such as metabolic syndromes or heart diseases remains controversial, despite a number of emerging studies. Several investigations have reported that eradication decreases the risk of dyslipidemia [19] or improved metabolic syndromes [20–22]. However, other studies have reported conflicting results [23] which might be originated from many confounding factors for these extraintestinal manifestations and the positivity of CagA (cytotoxicity associated gene A) toxin of *H. pylori*. age and sex/gender. When our team evaluated the effect of *H. pylori* on the metabolic parameters, high-density lipoprotein (HDL) increased in females after eradication but not in males. Body mass index increased after *H. pylori* eradication in males but not in females. Furthermore, *H. pylori* eradication was related to the decreasing of hemoglobin A1c (HbA1c) levels in patients with type 2 diabetes mellitus (T2DM) or prediabetes (preDM) over a long-term follow-up period, especially in males and subjects <65 years of age. Thus, in this chapter, the effect of *H. pylori* eradication on metabolic parameters and HbA1c levels are summarized in terms of sex/gender and age.

58.2 Metabolic Syndromes and *H. pylori* Infection

Metabolic syndrome is a constellation of metabolic disturbances, including hypertension, hyperglycemia, dyslipidemia, and central obesity, which has been reported to increase the risk of cardiovascular or cerebrovascular diseases [24]. According to the revised National Cholesterol Education Program (NCEP) criteria [25], an individual was diagnosed with metabolic syndrome if he or she had three or more of the following criteria: *(a)* abdominal obesity, waist circumference > 90 cm in men and > 80 cm in women using the International Obesity Task Force criteria for the Asian-Pacific population to determine waist circumference [26]; (b) triglyceride (TG) ≥ 150 mg/dL or medication use; (c) HDL–cholesterol (HDL-C) < 40 mg/dl in men and < 50 mg/dl in women or medication use; (d) blood pressure ≥ 130/85 mmHg or antihypertensive medication use; and (e) fasting glucose (FG) ≥ 100 mg/dL or medication use (insulin or oral agents). Recent reports showed a positive association between metabolic syndrome and *H. pylori* worldwide [27–30], but other studies have found conflicting results [31, 32].

58.2.1 The Effect of *H. pylori* on the Metabolic Parameters Depending on Age and Sex/Gender

Our team reported that total cholesterol (TC), low-density lipoprotein–cholesterol (LDL-C), and HDL-C were significantly different between subjects with and those without *H. pylori* seropositivity and significantly associated with *H. pylori* infection, even after adjusting for age, sex, and social factors such as household income and education, but TG and FG were not [27]. These findings of lipid profiles and FG are in agreement with the results of our previous study in 2019 [33] and other large-scale (> 13,000 subjects) studies from South Korea [34–36].

In terms of metabolic syndrome, our team reported that its prevalence was higher in *H. pylori*-seropositive individuals than in *H. pylori*-seronegative individuals (27.1% vs 21.0%, $p < 0.001$), and modeling of all the possible independent variables indicated that *H. pylori* seropositivity, as well as the expected risk factors—sex, age, smoking, and education level—significantly contributed to the development of metabolic syndrome [27] (Table 58.1). Furthermore, there was a significant increasing trend in the prevalence of *H. pylori* infection according to the increasing number of metabolic syndrome components, although this significance was marginally attenuated after adjusting for confounders [27]. This finding is concordant with the results of several other studies [2, 28, 29, 37–39] and *H. pylori* seropositivity significantly increased the prevalence of metabolic syndrome through lipid profile modification in a cross-sectional study of 7394 Japanese adults [29]. A systematic review and meta-analysis of 27,544 participants by Upala et al. also found a significant positive association between *H. pylori* infection and metabolic syndrome, with a pooled odds ratio (OR) of 1.34, compared with non-infected individuals [2]. However, several studies have reported conflicting results as well [31, 32, 40]. Naja et al. [31] reported no association between *H. pylori* seropositivity and metabolic syndrome among 308 Lebanese adults, and Takeoka et al. [32] observed no significant association between *H. pylori*-seropositive status and the risk of metabolic syndrome after adjusting for age and sex among 1044 Japanese individuals, while atrophic gastritis independently or together with *H. pylori* status significantly increased the risk of metabolic syndrome. Furthermore, another study [40] found that *H. pylori* seropositivity was not significantly associated with specific variables of metabolic syndrome based on the data of 31,311 American men in the Third National Health and Nutrition Examination Survey. East Asian and Middle Eastern countries exhibited a positive trend [28, 29, 37, 38, 41] However, several but not all studies with rather small sample sizes or studies conducted in Western countries exhibited a negative trend [31, 32, 42, 43]. These conflicting results between Asia and Western countries may be attributed to differences in host factors such as genetic obesity and diet, in addition to differences in toxic factors of *H. pylori*. Furthermore, this discrepancy could be explained by differences in the exclusion and inclusion criteria used for subject selection [11, 32, 40] or age and sex/gender. Actually, the positive association

Table 58.1 Univariate and multivariable regression analyses assessing the independent influence of *H. pylori* seropositivity on metabolic parameters (Adapted from Lim et al. [27])

	Univariate		Multivariable[a]	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
High BP[b]	1.349 (1.263–1.442)	<0.001	1.014 (0.941–1.092)	0.718
Abdominal obesity[c]	1.224 (1.141–1.312)	<0.001	1.018 (0.940–1.098)	0.636
FG ≥ 100 mg/dL	1.331 (1.247–1.421)	<0.001	1.011 (0.940–1.088)	0.768
TG ≥ 150 mg/dL	1.075 (0.992–1.164)	0.078	0.968 (0.888–1.056)	0.462
Low HDL-C[d]	1.347 (1.246–1.457)	<0.001	1.299 (1.196–1.412)	<0.001
High TC[e]	1.280 (1.141–1.435)	<0.001	1.317 (1.167–1.486)	<0.001
High LDL-C[f]	1.230 (1.150–1.315)	<0.001	1.205 (1.123–1.294)	<0.001
Obesity[g]	1.194 (1.112–1.283)	<0.001	1.048 (0.969–1.134)	0.242

OR odd ratio; *FG* fasting glucose; *TG* triglycerides; *HDL-C* high-density lipoprotein cholesterol; *TC* total cholesterol; *LDL-C* low-density lipoprotein cholesterol

[a] Multivariable model adjusted for age, gender, smoking, residence, household income, and educational level and the individual metabolic parameters

[b] BP ≥ 130/85 mmHg or antihypertensive medication use

[c] Waist circumference > 90 cm in men and > 80 cm in women

[d] HDL-C < 40 mg/dL in men and < 50 mg/dL in women

[e] TC ≥ 240 mg/dL

[f] LDL-C ≥ 130 mg/dL

[g] Body mass index ≥25 kg/m^2

between *H. pylori* and metabolic syndrome disappeared in old age in spite of increase of metabolic syndrome rates in *H. pylori* seropositive with age. For instance, Chen et al. [37] revealed that the *H. pylori* antibody was a predictor of metabolic syndrome among the subjects aged less than 50 years of age in 811 Taiwanese adults. In our study the association between *H. pylori* seropositivity and metabolic syndrome also disappeared in the ≥65 years old [27]. The reason why the rate of metabolic syndrome was not significantly different regardless of *H. pylori* serostatus among aged subjects might be that old age people tended to have other chronic diseases such as hypertension, hyperlipidemia, or diabetes which could contribute the development of metabolic syndrome. Korean study has shown the strong association between *H. pylori* seropositivity and mild, not moderate and not severe, coronary artery calcium score [44] suggesting that *H. pylori* infection may play a role in early atherosclerosis but not in the advanced stage. Usually, metabolic syndrome is higher in males than females (Table 58.2) but this difference disappeared in 70's [27] (Table 58.3). This might be originated from that estrogen protects the metabolic syndrome but long after menopause, this preventive effect disappeared. In addition, the South Korean annual prevalence trend of metabolic syndrome by sex from 2007 to 2018 nicely showed that it increased continuously in males from 22.5% to 27.5% but that of females rather decreased from 20.8% to 17.9% [45] (Fig. 58.1). Although the prevalence of *H. pylori* in males are continuously higher than that of females, it continuously decreased from 1998 (66.9%), 2005

Table 58.2 Proportion of sociodemographic factors and *H. pylori* serostatus in patients with or without metabolic syndrome (Adapted from Lim et al. [27])

		Metabolic syndrome (+)			Metabolic syndrome (−)			
		n	Prevalence (%)	Proportion (%)	*n*	Prevalence (%)	Proportion (%)	*p*-value
All		3598	23.7		11,597			
Sex	Male	2680	33.1	74.5	5409	66.9	46.6	<0.001
	Female	918	12.9	25.5	6188	87.1	53.4	
Mean age (years)†		54.7 ± 10.9			49.5 ± 11.3			<0.001
Age group (years)	16–19	1	6.3	0.03	15	93.8	0.13	<0.001*
	20–29	26	5.3	0.7	465	94.7	4.0	
	30–39	260	13.2	7.2	1710	86.8	14.7	
	40–49	873	19.5	24.3	3611	80.5	31.1	
	50–59	1316	26.2	36.6	3709	73.8	32.0	
	60–69	767	32.2	21.3	1613	67.8	13.9	
	≥70	355	42.8	9.9	474	57.2	4.1	
BMI (kg/m^2)†		26.1 ± 2.9			22.4 ± 2.8			<0.001
H. pylori serostatus	Positive	1789	27.2	49.7	4780	72.8	41.2	<0.001
	Negative	1809	21.0	50.3	6817	79.0	58.8	
Smoking status	Nonsmoker	2278	19.9	63.3	9141	80.1	78.8	<0.001
	Ex-smoker	839	34.8	23.3	1571	65.2	13.6	
	Current smoker	481	35.2	13.4	885	64.8	7.6	
Household income‡	Low	155	23.5	4.5	504	76.5	4.6	0.09
	Medium	1313	22.9	37.9	4411	77.1	39.9	
	High	1994	24.5	57.6	6130	75.5	55.5	
Educational level§	Low	158	42.7	4.4	212	57.3	1.8	<0.001
	Medium	506	27.3	14.1	1348	72.7	11.7	
	High	2924	22.6	81.5	10,003	77.4	86.5	
Residence	Seoul	2246	22.1	62.4	7913	77.9	68.2	<0.001
	Provinces	1352	26.8	37.6	3684	73.2	31.8	

BMI body mass index; †mean ± standard deviation; * trend *p*-value; ‡household income was classified as low (less than US $ 3000), medium (US $ 3000–10,000), or high (more than US $ 10,000); § educational level was classified as low (middle school graduates or less), medium (high school graduates or university dropouts), or high (university graduates or graduates of a postgraduate course)

(59.6%), 2011 (54.4%) to 2016–2017 (43.9%) in Korea [46]. Thus, metabolic syndrome in males might be originated from the other factors such as diet and obesity instead of *H. pylori* infection.

Table 58.3 Age-specific prevalence of metabolic syndrome by sex (Adapted from Lim et al. [27])

		Male (*n*, %)	Female (*n*, %)	*p*-value
Age groups (years old)	16–19	1 (14.2)	0 (0)	0.89
	20–29	20 (9.2)	6 (2.2)	0.00106
	30–39	241 (25.0)	19 (1.8)	<0.001
	40–49	718 (31.7)	155 (7.0)	<0.001
	50–59	997 (35.3)	319 (14.5)	<0.001
	60–69	509 (38.1)	258 (24.7)	<0.001
	≥70	192 (40.0)	163 (46.7)	0.063

Variables are presented as numbers (%)

58.2.2 The Underlying Mechanisms of *H. pylori* on the Metabolic Parameters

What are the pathomechanisms underlying in this association between *H. pylori* infection and metabolic syndrome? Although the clear mechanism remains to be elucidated, various potential mechanisms linking *H. pylori* infection to each component of metabolic syndrome have been postulated. First, regarding the pathogenic mechanisms proposed to associate *H. pylori* infection with atherogenic dyslipidemia, experimental investigations demonstrated that chronic bacterial infection, such as *H. pylori* infection, induces production of pro-inflammatory cytokines, such as interleukins (ILs) 1 and 6, interferon-alpha, and tumor necrosis factor-alpha, which may alter lipid metabolism in a variety of ways [27]. These cytokines decrease the activation of adipose tissue lipoprotein lipase, provoke hepatic fatty acid synthesis, influence lipolysis, and stimulate hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity [47–49].

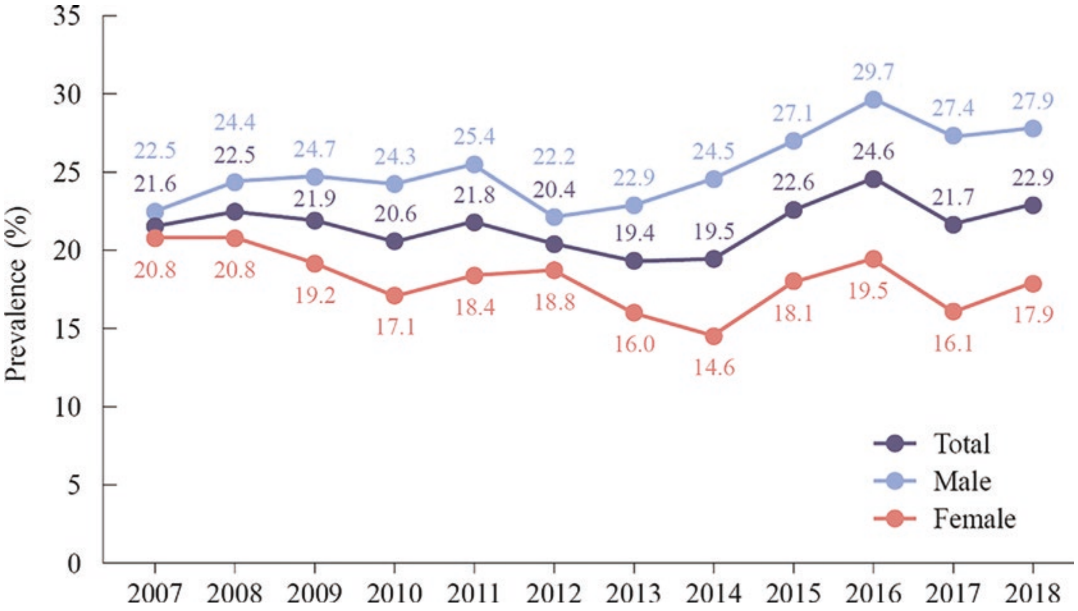

Fig. 58.1 Annual prevalence trend of metabolic syndrome by sex from 2007 to 2018 (adapted from Huh et al. [45])

58.2.3 The Effect of *H. pylori* Eradication on the Metabolic Parameters

The effect of *H. pylori* eradication on metabolic syndrome remains controversial. Several investigations have reported that *H. pylori* eradication decreases the risk of dyslipidemia [19] or improved metabolic syndrome [20–22]. However, studies regarding this topic have reported conflicting results. In addition, there was no long-term follow-up study.

58.2.3.1 Different Effects of *H. pylori* Eradication on the Metabolic Parameters

A systemic review and meta-analysis published in 2017 showed that *H. pylori* eradication did not improve insulin resistance (IR), TG, HDL-C, LDL-C, or FG, but increased body weight and body mass index (BMI) [50]. However, this study included 3–4 studies for each value [50]. In contrast, a multicentre, open-label, randomised clinical trial (RCTs) done at nine medical centers in Taiwan including 1620 adults showed improvements in metabolic parameters, with a decrease in IR, TG, and LDL and an increase in HDL, without significant change in the prevalence of metabolic syndrome at week 8 and 1 year after *H. pylori* eradication [51]. As still there have been

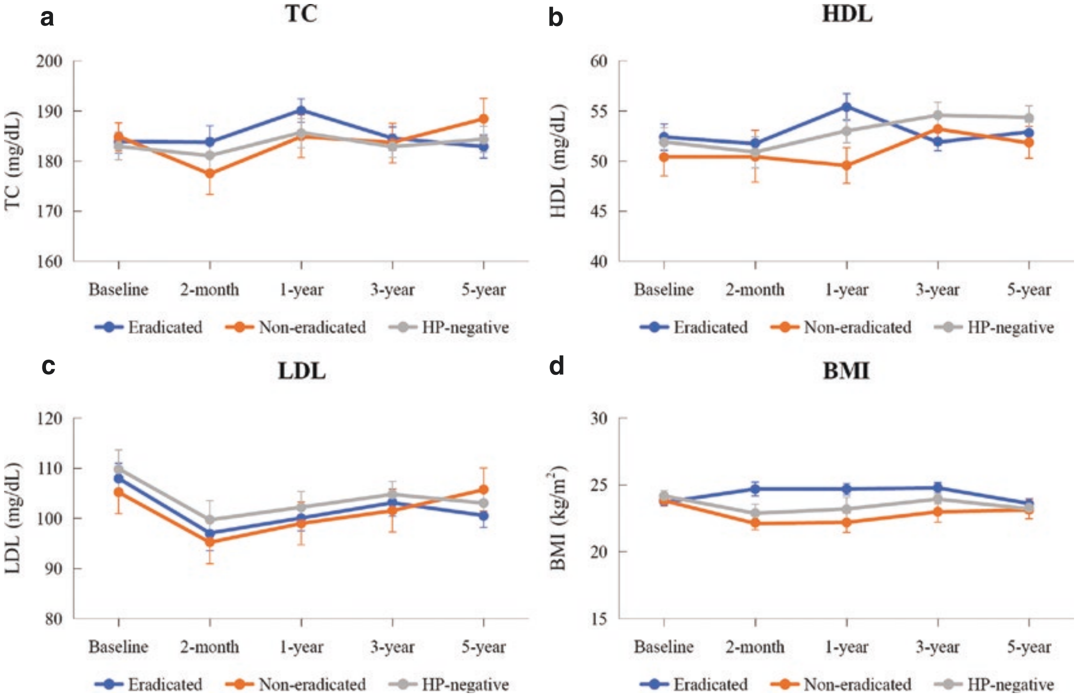

Fig. 58.2 Changes in the HDL and BMI levels over time in patients in the *H. pylori*-uninfected, *H. pylori*-non-eradicated, and *H. pylori*-eradicated groups depending on sex (Adapted from Park et al. [23]). Serial changes in TC (**a**), HDL (**b**), LDL (**c**), and BMI (**d**) at baseline and after 2 months and one, three, and 5 years of follow-up in the *H. pylori*-negative, *H. pylori*-non-eradicated, and *H. pylori*-eradicated groups. The overall trends of HDL levels were different among the three groups throughout the 5 years of follow-up with marginal significance, and the difference was greatest at 1 year (**b**). The overall trends of BMI were significantly different among groups, and the difference was greatest at 1 year as well (**d**). There was no significant difference in the TC and LDL change over 5 years among the *H. pylori*-uninfected, *H. pylori*-non-eradicated, and *H. pylori*-eradicated groups (**a**) (**c**). *TC* total cholesterol; *HDL* high-density lipoprotein; *LDL* low-density lipoprotein; *BMI* body mass index

controversial results the effect of *H. pylori* eradication on metabolic syndrome might differ depending on sex, ethnicity, diet, exercise, or BMI. Few studies have investigated the long-term outcomes in the effects of *H. pylori* eradication therapy on metabolic syndrome.

Our team reported that the metabolic parameters during follow-up among *H. pylori*-uninfected ($n = 509$), *H. pylori*-non-eradicated ($n = 346$), and *H. pylori*-eradicated ($n = 666$) groups showed that HDL (Fig. 58.2b) and BMI increased after eradication (Fig. 58.2d), with a significant difference at 1 year of follow-up [23]. However, there was no change in the TC (Fig. 58.2a) and LDL (Fig. 58.2c). In females, HDL increased after *H. pylori* eradication ($p = 0.023$) (Fig. 58.3c), and BMI increased after *H. pylori* eradication in male subjects ($p = 0.010$) [23] (Fig. 58.3b). However, there was no significant difference of HDL in males (Fig. 58.3a), and BMI in females [23] (Fig. 58.3d). After propensity score matching, the HDL change in female remained significant, but the statistical significance of BMI change in male group disappeared ($p = 0.089$) [23]. These results suggest that *H. pylori* eradication affected metabolic parameters differently depending on sex. Why was the influence of *H. pylori* eradication on HDL and BMI maximized at 1 year and decreased after that? This may be a result of the gradual normalization of cytokines after *H. pylori* eradication. Ando et al. reported that IL-6 activity showed 6 months of gradual normalization compared to

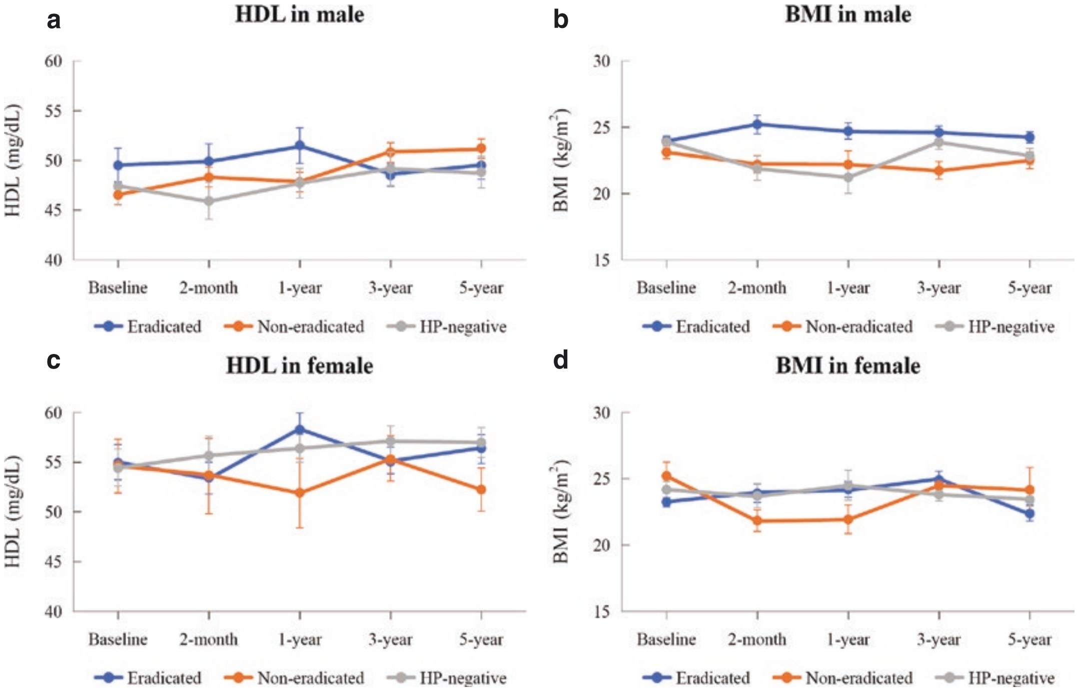

Fig. 58.3 Changes in metabolic parameters over time in male and female subjects depending on *H. pylori* eradication (Adapted from Park et al. [23]). Serial changes in HDL in males (**a**), BMI in males (**b**), HDL in females (**c**), and BMI in females (**d**) at baseline and after 2 months, one, three, and 5 years of follow-up in the *H. pylori*-uninfected, *H. pylori*-non-eradicated, and *H. pylori*-eradicated groups. The overall trends of the BMI levels in males (**b**) and HDL levels in females (**c**) were significantly different among the three groups. There was no significant difference in the changes in HDL levels in males and BMI levels in females over the 5-year follow-up period among the groups. *HDL* high-density lipoprotein; *BMI* body mass index

the 1-month rapid normalization of IL-8 after *H. pylori* eradication [52]. In addition, HDL may be affected by various factors, including lifestyle changes and body weight. Actually, BMI increased after *H. pylori* eradication although HDL increased, which is contradictory considering that increased body weight generally has a negative effect on lipid profile in our study [23]. Thus, it can be hypothesized that cytokines normalized gradually after *H. pylori* eradication, and its positive effect on metabolic parameters was strong enough to overcome the negative effect of body weight increase until 1 year of follow-up [23].

58.2.3.2 The Underlying Mechanisms of Different Effects of Sex on the *H. pylori* Eradication-Induced Metabolic Parameters

In terms of the sex differences regarding different effects of *H. pylori* eradication-induced metabolic parameters, there was no definite explanation. However, sex hormones could be related to these results. It is well known that sex hormones have a substantial influence on lipid metabolism. Premenopausal females have higher HDL and lower LDL levels than males. Lipid profile changes in females after menopause can be partially reversed by exogenous hormone replacement therapy [53]. Furthermore, Godsland et al. reported that hormone replacement therapy in healthy postmenopausal females raised HDL cholesterol and lowered LDL and TC, although these effects differed by estrogen route and progesterone type [54]. A recent meta-analysis showed that the TG, TC, LDL, and TC to HDL ratio levels were significantly higher in postmenopausal females than in premenopausal females [55]. Thus, sex hormones could induce sex difference of the *H. pylori* eradication effect on lipid profiles although sex hormones may not be solely responsible for sex differences in terms of BMI or obesity. According to the previous studies, obesity was more prevalent among males in some countries such as Japan, Korea, China, Germany, France, the United Kingdom, and the United States, in contrast to the worldwide average data [18, 56]. Khang et al. suggested that the sex difference in obesity in East Asian countries may be attributed to social factors such as increased interest in leanness in Asian females [18, 57].

58.3 The Effects of *H. pylori* and its Eradication on the Insulin Resistance or Hemoglobin A1c Depending on Sex/Gender and Age

Similar to the metabolic parameters, several studies have reported conflicting results regarding the effects of *H. pylori* on the IR or HbA1c [31, 40]. Naja et al. [31] reported no association between *H. pylori* seropositivity and IR among 308 Lebanese adults. Furthermore, Gillum [40] suggested that *H. pylori* seropositivity was not significantly associated with specific variables of IR syndrome, based on the data of 31,311 American men in the Third National Health and Nutrition Examination Survey. In contrast, there was a potential association between *H. pylori* infection and IR in a systematic review of 2120 participants [39]. With regards to the relation between *H. pylori* infection and IR, a plausible explanation is that *H. pylori* infection causes impairment of hepatic IR via the c-Jun/microRNA-203/suppressor of cytokine signaling 3 pathway [58]. In addition, the role of cytokines in IR has been suggested; i.e., inflammatory cytokines lead to the phosphorylation of serine/threonine residues on the insulin receptor, which prevents its activation and thus decreases insulin sensitivity [59]. In terms of the effect of *H. pylori* eradication, there were several reports that HbA1c levels decreased in patients with T2DM or preDM in short-term [60–62] and its improvement was sustained over long-term follow-up [5].

58.3.1 The Effects of *H. pylori* on the Insulin Resistance or Glycosylated Hemoglobin A1c

Several studies suggested a relationship between *H. pylori* infection and IR and DM [29, 39, 63]. Considerable data supported that *H. pylori* infection could induce pathogenic mechanisms of

DM, such as IR and inflammation [64], and that *H. pylori* infection was associated with the occurrence of DM and the increased level of HbA1c [30, 65]. The most attracting mechanism is regarding ghrelin. That is, ghrelin, which is involved in ingestion, appetite, and nutrition, especially lipid absorption and lipogenesis [66], was significantly lower in *H. pylori*-positive than in *H. pylori*-negative individuals [67]. This phenomenon is explained that *H. pylori* infection can impair the synthesis of ghrelin [68]. As ghrelin can regulate insulin sensitivity and stimulate insulin-induced glucose uptake [69], low plasma ghrelin levels are associated with elevated fasting plasma glucose levels and IR [70]. It means that *H. pylori* infection can provoke IR and DM. In addition, *H. pylori* infection is possibly be associated with impaired hepatic IR through the c-Jun/microRNA-203/suppressor of the cytokines that signal three pathways similar to the explanation of metabolic parameters [58]. In addition, inflammatory cytokines induce the phosphorylation of serine/threonine residues in the insulin receptor, preventing its activation and reducing insulin sensitivity [59].

58.3.2 The Effects of *H. pylori* Eradication on the Insulin Resistance or Hemoglobin A1c and the Possible Underlying Mechanism

Recently, several studies have reported decreases of FG and HbA1c levels after 3 or 6 months after *H. pylori* eradication [60–62, 71, 72]. For instance, Dogan et al. reported significant changes of FG and HbA1c level between baseline and 6 months after *H. pylori* eradication in subjects with normal glucose levels [71]. In addition, in 319 patients with T2DM, Cheng et al. demonstrated that the eradicated group had significant within-group and between-group improvements in HbA1c, compared with the non-eradicated group [72]. Actually, there must be an underlying mechanism for this improvement. First, *H. pylori* eradication upregulated preproghrelin mRNA expression and increases plasma acyl ghrelin levels, contributing to the abatement of postprandial distress symptoms in functional dyspepsia [73]. Another explanation involves glucagon-like peptide-1 (GLP-1) and the gut microbiota. GLP-1 regulates glucose homeostasis [74], has trophic effects on pancreatic beta cells [75], reduces acid secretion in the stomach, and delays gastric emptying. It also regulates appetite by inducing a feeling of satiety. Specific bacteria, including *Bifidobacterium* spp., *Lactobacillus* spp., and *Akkermansia muciniphila* spp., contribute to the regulation of incretin hormones such as GLP-1 [76]. *H. pylori* eradication treatment could improve carbohydrate metabolism by increasing GLP-1 secretion, and this change is closely related to alterations in the gut microbiota [60, 77], even after a long-term follow-up [51]. These underlying mechanisms support our observation of the effect of *H. pylori* eradication on the decrease of in HbA1c levels over a long-term follow-up. This provides new insights into the role of *H. pylori* eradication and supports the rationale for the recommendation of *H. pylori* eradication therapy.

58.3.3 The Effects of *H. pylori* Eradication on the Hemoglobin A1c Depending on Age and Sex

When our team evaluated the long-term effect of *H. pylori* eradication on the glycemic control in the 124 asymptomatic subjects with T2DM or preDM [5], the HbA1c levels significantly decreased in the eradicated group compared to the negative group and the non-eradicated groups (at the first year, $p = 0.024$; at the fifth year, $p = 0.009$) [5] (Fig. 58.4a). Especially, the HbA1c levels decreased in male, and/or subjects <65 years of age in subgroup analyses. This positive association in younger age is consistent with the results of earlier research on the relationship between *H. pylori* infection and metabolic syndrome. Chen et al. observed that subjects with metabolic syndrome <50 years of age had a higher prevalence of *H. pylori* infection [37]. Similarly, our team demonstrated that the association between *H. pylori* seropositivity and metabolic syndrome was present in subjects

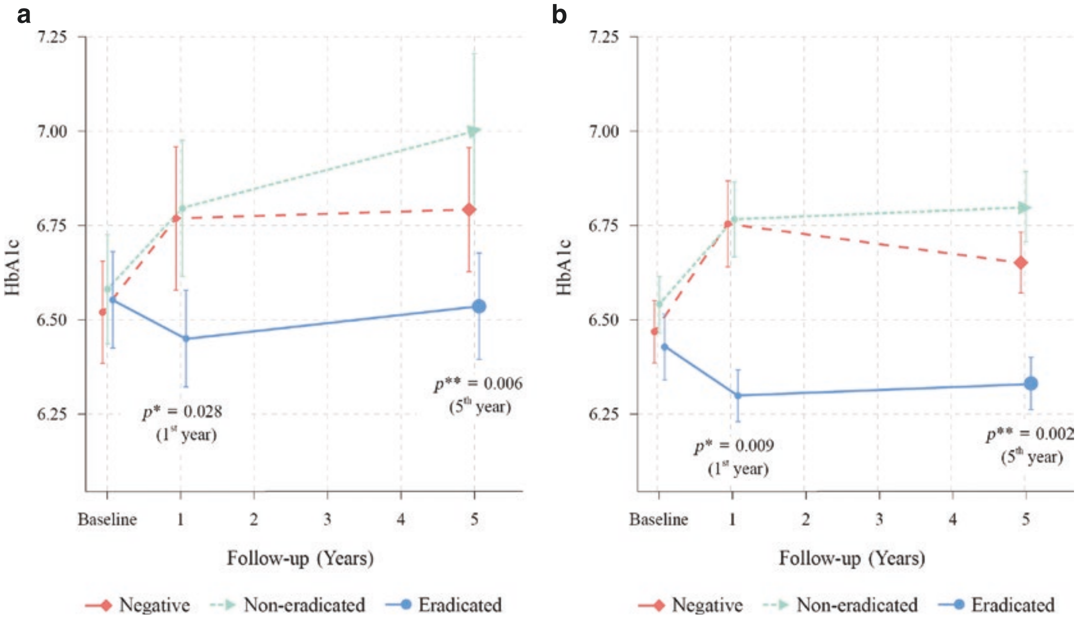

Fig. 58.4 HbA1c changes over time in each group (Adapted from Kim et al. [5]). (**a**) Analysis of the entire subjects and (**b**) Analysis except for patients who had hypoglycemic agent changes during follow-up. Mean HbA1c (A1C) values of each group are demonstrated. Improvements in A1C after HP eradication at the first year of follow-up were observed, and these changes were maintained until the fifth year of follow-up, in both (**a**) and (**b**). Note: The *p* values were calculated using linear mixed model. *Adjusted *p* value, at the first year of follow-up, †adjusted *p* value, at the fifth year of follow-up. Adjusted variables; age, sex, smoke, alcohol consumption, hypertension, and dyslipidemia

<65 years of age but disappeared in the older ones [27]. Park et al. reported that the effect of *H. pylori* eradication on the risk of dyslipidemia was more evident in subjects <50 years of age [19]. Altogether, metabolic syndrome prevalence was not significantly different regardless of the *H. pylori* serological status in older subject, they tend to have other chronic diseases, such as hypertension, hyperlipidemia, or diabetes, contributing to the development of metabolic syndrome. Regardless of the geographic location, the *H. pylori* infection in most individuals is acquired during early childhood [78]. This chronic *H. pylori* infection plays an important role in the development of adulthood but this systemic inflammation caused by *H. pylori* infection may influence metabolic syndrome development somewhat at an early stage, but not in an advanced stage or old age [79]. Thus, an early eradication of *H. pylori* is more beneficial in terms of prevention of metabolic syndrome or T2DM or preDM.

In addition, a correlation between sex and HbA1c levels, specifically in male subjects, is compatible with previous studies that showed male subjects are more affected by *H. pylori* infection than female subjects, such as higher gastric inflammation and activity scores in the antrum with *H. pylori* infection [80]. According to a large dataset including worldwide mortality and prevalence of cancers, the incidence of gastric cancer in male subjects was about twice as high as in female individuals [81]. Interestingly, in a mouse-model study, severe dysplasia and gastric carcinogenesis predominated in male subjects infected with *H. pylori* [82]. Furthermore, metabolic syndrome was higher in male subjects, except in the age-group of >70 years, and the metabolic syndrome in female subjects after definite menopause was higher than in male subjects [27]. Similarly, the *H. pylori* infection increased the risk of metabolic syndrome in female subjects aged ≥50 years [83]. In addition, *H. pylori* infection was a statistically significant predictor of DM incidence in male subjects [30].

Again, the underlying mechanism is related to estrogen. Ovarian-dependent female hormones, such as estradiol (E2), prevented gastric carcinogenesis and inflammation of the gastric tissue in INS-GAS mice infected with *H. pylori*. In addition, exogenous E2 supplementation has shown protective effects against the development of *H. pylori*-induced gastritis and premalignant lesions through several mechanisms [84]. In the relationship between metabolic syndrome and E2, recent researches showed that TC, LDL-C, and TG were higher in postmenopausal female subjects than in premenopausal females [55]. In addition, hormone therapy with estrogen alone has been associated with decreased TC and LDL-C levels and elevated HDL-C levels [54].

58.4 Conclusions

Although *H. pylori* mainly colonize in the gastric mucosa, this bug is associated with many extraintestinal manifestations such as endocrine, cardiovascular, hematologic, neurologic, and autoimmune diseases. Metabolic syndrome, which is characterized by clusters of metabolic abnormalities, has been highlighted as a risk factor for various cardiovascular diseases, and T2DM or preDM. These *H. pylori* infection related diseases differently manifest, depending on age and sex/gender that the effect of *H. pylori* eradication also could be different. However, the effect of *H. pylori* eradication on the extraintestinal diseases remains controversial. These differences might be related with that the development of these extraintestinal manifestations might be associated with sex hormones. For instance, HDL increased in females after *H. pylori* eradication but not in males. Body mass index increased 1 year after *H. pylori* eradication in males but not females. Furthermore, *H. pylori* eradication was related to the decreasing of HbA1c levels in patients with T2DM or preDM over a long-term follow-up period, especially in males and subjects <65 years of age. Thus, it is necessary to figure out the role of sex/gender as well as the age in terms of prevention of these diseases by early eradication of *H. pylori*.

References

1. Suerbaum S, Michetti P. *Helicobacter pylori* infection. N Engl J Med. 2002;347:1175–86.
2. Upala S, Jaruvongvanich V, Riangwiwat T, Jaruvongvanich S, Sanguankeo A. Association between *Helicobacter pylori* infection and metabolic syndrome: a systematic review and meta-analysis. J Dig Dis. 2016;17:433–40.
3. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global prevalence of *Helicobacter pylori* infection: systematic review and meta-analysis. Gastroenterology. 2017;153:420–9.
4. Papamichael KX, Papaioannou G, Karga H, Roussos A, Mantzaris GJ. *Helicobacter pylori* infection and endocrine disorders: is there a link? World J Gastroenterol. 2009;15:2701–7.
5. Kim WS, Choi Y, Kim N, Lim SH, Noh G, Kim KW, et al. Long-term effect of the eradication of *Helicobacter pylori* on the hemoglobin A1c in type 2 diabetes or prediabetes patients. Korean J Intern Med. 2022;37:579–90.
6. Wang B, Yu M, Zhang R, Chen S, Xi Y, Duan G. A meta-analysis of the association between *Helicobacter pylori* infection and risk of atherosclerotic cardiovascular disease. Helicobacter. 2020;25:e12761.
7. Alvarez-Arellano L, Maldonado-Bernal C. *Helicobacter pylori* and neurological diseases: married by the laws of inflammation. World J Gastrointest Pathophysiol. 2014;5:400–4.
8. Lim JH, Kim N, Lim SH, Kwon JW, Shin CM, Chang YS, et al. Inverse relationship between *Helicobacter Pylori* infection and asthma among adults younger than 40 years: A cross-sectional study. Medicine (Baltimore). 2016;95:e2609.
9. Leontiadis GI, Sharma VK, Howden CW. Non-gastrointestinal tract associations of *Helicobacter pylori* infection. Arch Intern Med. 1999;159:925–40.
10. Franceschi F, Gasbarrini A, Polyzos SA, Kountouras J. Extragastric diseases and Helicobacter pylori. Helicobacter. 2015;20 Suppl 1:40–6.
11. Watanabe S, Hojo M, Nagahara A. Metabolic syndrome and gastrointestinal diseases. J Gastroenterol. 2007;42:267–74.
12. Jinjuvadia R, Antaki F, Lohia P, Liangpunsakul S. The association between nonalcoholic fatty liver disease and metabolic abnormalities in the United States population. J Clin Gastroenterol. 2017;51:160–6.
13. Mozumdar A, Liguori G. Persistent increase of prevalence of metabolic syndrome among US adults: NHANES III to NHANES 1999–2006. Diabetes Care. 2011;34:216–9.
14. Nestel P, Lyu R, Low LP, Sheu WH, Nitiyanant W, Saito I, et al. Metabolic syndrome: recent prevalence

in East and Southeast Asian populations. Asia Pac J Clin Nutr. 2007;16:362–7.

15. Lim S, Shin H, Song JH, Kwak SH, Kang SM, Yoon JW, et al. Increasing prevalence of metabolic syndrome in Korea: the Korean national health and nutrition examination survey for 1998–2007. Diabetes Care. 2011;34:1323–8.
16. Oh SW. Obesity and metabolic syndrome in Korea. Diabetes Metab J. 2011;35:561–6.
17. Tran BT, Jeong BY, Oh JK. The prevalence trend of metabolic syndrome and its components and risk factors in Korean adults: results from the Korean National Health and nutrition examination survey 2008–2013. BMC Public Health. 2017;17:71.
18. Lee SE, Han K, Kang YM, Kim SO, Cho YK, Ko KS, et al. Trends in the prevalence of metabolic syndrome and its components in South Korea: findings from the Korean National Health Insurance Service Database (2009–2013). PLoS One. 2018;13:e0194490.
19. Park Y, Kim TJ, Lee H, Yoo H, Sohn I, Min YW, et al. Eradication of *Helicobacter pylori* infection decreases risk for dyslipidemia: A cohort study. Helicobacter. 2021;26:e12783.
20. Iwai N, Okuda T, Oka K, Hara T, Inada Y, Tsuji T, et al. *Helicobacter pylori* eradication increases the serum high density lipoprotein cholesterol level in the infected patients with chronic gastritis: A single-center observational study. PLoS One. 2019;14:e0221349.
21. Jun W, Hamasaki M, Kotani K. The effect of *Helicobacter pylori* eradication on lipid levels: A meta-analysis. J Clin Med. 2021;29:153–9.
22. Dachi K, Mishiro T, Toda T, Kano N, Fujihara H, Mishima Y, et al. Effects of *Helicobacter pylori* eradication on serum lipid levels. J Clin Biochem Nutr. 2018;62:264–9.
23. Park J, Kim N, Kim WS, Lim SH, Choi Y, Jo HH, et al. Long-term effects of the eradication of *Helicobacter pylori* on metabolic parameters, depending on sex, in South Korea. Gut Liver. 2023;17:58–68.
24. Eckel R, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365:1415–28.
25. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005;112:2735–52.
26. World Health Organization. Regional Office for the Western Pacific. The Asia-Pacific perspective: redefining obesity and its treatment. Sydney: Health Communications Australia; 2000. p. 15–21. http://www.who.int/iris/handle/10665/206936. Accessed 05 Jan 2023.
27. Lim SH, Kim N, Kwon JW, Kim SE, Baik GH, Lee JY, et al. Positive association between *Helicobacter pylori* infection and metabolic syndrome in a Korean population: A multicenter nationwide study. Dig Dis Sci. 2019;64:2219–30.
28. Chen TP, Hung HF, Chen MK, Lai HH, Hsu WF, Huang KC, et al. *Helicobacter pylori* infection is positively associated with metabolic syndrome in Taiwanese adults: a Cross-sectional study. Helicobacter. 2015;20:184–91.
29. Gunji T, Matsuhashi N, Sato H, Fujibayashi K, Okumura M, Sasabe N, et al. *Helicobacter pylori* infection is significantly associated with metabolic syndrome in the Japanese population. Am J Gastroenterol. 2008;103:3005–10.
30. Chen YY, Fang WH, Wang CC, Kao TW, Chang YW, Wu CJ, et al. *Helicobacter pylori* infection increases risk of incident metabolic syndrome and diabetes: A cohort study. PLoS One. 2019;14:e0208913.
31. Naja F, Nasreddine L, Hwalla N, Moghames P, Shoaib H, Fatfat M, et al. Association of *H. pylori* infection with insulin resistance and metabolic syndrome among Lebanese adults. Helicobacter. 2012;17:444–51.
32. Takeoka A, Tayama J, Yamasaki H, Kobayashi M, Ogawa S, Saigo T, et al. Impact of *Helicobacter pylori* immunoglobulin G levels and atrophic gastritis status on risk of metabolic syndrome. PLoS One. 2016;11:e0166588.
33. Lim SH, Kwon JW, Kim N, Kim GH, Kang JM, Park MJ, et al. Prevalence and risk factors of *Helicobacter pylori* infection in Korea: nationwide multicenter study over 13 years. BMC Gastroenterol. 2013;13:104.
34. Sung KC, Rhee EJ, Ryu SH, Beck SH. Prevalence of *Helicobacter pylori* infection and its association with cardiovascular risk factors in Korean adults. Int J Cardiol. 2005;102:411–7.
35. Nam SY, Ryu KH, Park BJ, Park S. Effects of *Helicobacter pylori* infection and its eradication on lipid profiles and cardiovascular diseases. Helicobacter. 2015;20:125–32.
36. Kim TJ, Lee H, Kang M, Kim JE, Choi YH, Min YW, et al. *Helicobacter pylori* is associated with dyslipidemia but not with other risk factors of cardiovascular disease. Sci Rep. 2016;6:38015.
37. Chen LW, Chien CY, Yang KJ, Kuo SF, Chen CH, Chien RN. *Helicobacter pylori* Infection increases insulin resistance and metabolic syndrome in residents younger than 50 years old: a community-based study. PLoS One. 2015;10:e0128671.
38. Shin DW, Kwon HT, Kang JM, Park JH, Choi HC, Park MS, et al. Association between metabolic syndrome and *Helicobacter pylori* infection diagnosed by histologic status and serological status. J Clin Gastroenterol. 2012;46:840–5.
39. Polyzos SA, Kountouras J, Zavos C, Deretzi G. The association between *Helicobacter pylori* infection and insulin resistance: a systematic review. Helicobacter. 2011;16:79–88.
40. Gillum RF. Infection with *Helicobacter pylori*, coronary heart disease, cardiovascular risk factors, and systemic inflammation: the Third National Health and Nutrition Examination Survey. J Natl Med Assoc. 2004;96:1470–6.
41. Nabipour I, Vahdat K, Jafari SM, Pazoki R, Sanjdideh Z. The association of metabolic syndrome and *Chlamydia pneumoniae*, *Helicobacter pylori*, cyto-

megalovirus, and herpes simplex virus type 1: the Persian Gulf Healthy Heart Study. Cardiovasc Diabetol. 2006;5:25.

42. Malamug LR, Karnchanasorn R, Samoa R, Chiu KC. The role of *Helicobacter pylori* seropositivity in insulin sensitivity, beta cell function, and abnormal glucose tolerance. Scientifca (Cairo). 2014;2014:870165.
43. Gerig R, Ernst B, Wilms B, Thurnheer M, Schultes B. Gastric *Helicobacter pylori* infection is associated with adverse metabolic traits in severely obese subjects. Obesity (Silver Spring). 2013;21:535–7.
44. Park MJ, Choi SH, Kim D, Kang SJ, Chung SJ, Choi SY, et al. Association between *Helicobacter pylori* seropositivity and the coronary artery calcium score in a screening population. Gut Liver. 2011;5:321–7.
45. Huh JH, Kang DR, Kim JY, Koh KK. Metabolic syndrome fact sheet 2021: Executive report. Cardiometab Syndr J. 2021;1:125–34.
46. Lim SH, Kim N, Kwon JW, Kim SE, Baik GH, Lee JY, et al. Trends in the seroprevalence of *Helicobacter pylori* infection and its putative eradication rate over 18years in Korea: a cross-sectional nationwide multicenter study. PLoS One. 2018;13:e0204762.
47. Gallin JI, Kaye D, O'Leary WM. Serum lipids in infection. N Engl J Med. 1969;281:1081–6.
48. Feingold KR, Grunfeld C. Role of cytokines in inducing hyperlipidemia. Diabetes. 1992;41:97–101.
49. Memon RA, Grunfeld C, Moser AH, Feingold KR. Tumor necrosis factor mediates the efects of endotoxin on cholesterol and triglyceride metabolism in mice. Endocrinology. 1993;132:2246–53.
50. Upalaa S, Sanguankeoa A, Saleema SA, Jaruvongvanich V. Effects of *Helicobacter pylori* eradication on insulin resistance and metabolic parameters: a systematic review and meta-analysis. Eur J Gastroenterol Hepatol. 2017;29:153–9.
51. Liou JM, Chen CC, Chang CM, Fang YJ, Bair MJ, Chen PY, et al. Long-term changes of gut microbiota, antibiotic resistance, and metabolic parameters after *Helicobacter pylori* eradication: a multicentre, open-label, randomised trial. Lancet Infect Dis. 2019;19:1109–20.
52. Ando T, Kusugami K, Ohsuga M, Ina K, Shinoda M, Konagaya T, et al. Differential normalization of mucosal interleukin-8 and interleukin-6 activity after *Helicobacter pylori* eradication. Infect Immun. 1998;66:4742–7.
53. Link JC, Reue K. The genetic basis for sex differences in obesity and lipid metabolism. Annu Rev Nutr. 2017;37:225–45.
54. Godsland IF. Effects of postmenopausal hormone replacement therapy on lipid, lipoprotein, and apolipoprotein (a) concentrations: analysis of studies published from 1974-2000. Fertil Steril. 2001;75:898–915.
55. Ambikairajah A, Walsh E, Cherbuin N. Lipid profile differences during menopause: a review with meta-analysis. Menopause. 2019;26:1327–33.
56. Kim KB, Shin YA. Males with obesity and overweight. J Obes Metab Syndr. 2020;29:18–25.
57. Khang YH, Yun SC. Trends in general and abdominal obesity among Korean adults: Findings from 1998, 2001, 2005, and 2007 Korea national health and nutrition examination surveys. J Korean Med Sci. 2010;25:1582–8.
58. Zhou X, Liu W, Gu M, Zhou H, Zhang G. *Helicobacter pylori* infection causes hepatic insulin resistance by the c-Jun/miR-203/SOCS3 signaling pathway. J Gastroenterol. 2015;50:1027–40.
59. Wellen KE, Hotamisligil GS. Infammation, stress, and diabetes. J Clin Invest. 2005;115:1111–9.
60. Cornejo-Pareja I, Martín-Núñez GM, Roca-Rodríguez MM, Cardona F, Coin-Aragüez L, Sánchez-Alcoholado L, et al. *H. pylori* eradication treatment alters gut microbiota and GLP-1 secretion in humans. J Clin Med. 2019;8:451.
61. Martín-Núñez GM, Cornejo-Pareja I, Coin-Aragüez L, Roca-Rodríguez MDM, Muñoz-Garach A, Clemente-Postigo M, et al. *H. pylori* eradication with antibiotic treatment causes changes in glucose homeostasis related to modifications in the gut microbiota. PLoS One. 2019;14:e0213548.
62. Mokhtare M, Mirfakhraee H, Arshad M, Samadani Fard SH, Bahardoust M, Movahed A, et al. The effects of *Helicobacter pylori* eradication on modification of metabolic syndrome parameters in patients with functional dyspepsia. Diabetes Metab Syndr. 2017;11 Suppl 2:S1031–5.
63. Simon L, Tornoczky J, Toth M, Jambor M, Sudar Z. The significance of *Campylobacter pylori* infection in gastroenterologic and diabetic practice. Orv Hetil. 1989;130:1325–9.
64. Kayar Y, Pamukcu O, Eroglu H, Kalkan Erol K, Ilhan A, Kocaman O. Relationship between *Helicobacter pylori* infections in diabetic patients and inflammations, metabolic syndrome, and complications. Int J Chronic Dis. 2015;2015:290128.
65. Bajaj S, Rekwal L, Misra SP, Misra V, Yadav RK, Srivastava A. Association of *Helicobacter pylori* infection with type 2 diabetes. Indian J Endocrinol Metab. 2014;18:694–9.
66. Chabot F, Caron A, Laplante M, St-Pierre DH. Interrelationships between ghrelin, insulin and glucose homeostasis: physiological relevance. World J Diabetes. 2014;5:328–41.
67. Gao XY, Kuang HY, Liu XM, Duan P, Yang Y, Ma ZB. Circulating ghrelin/obestatin ratio in subjects with *Helicobacter pylori* infection. Nutrition. 2009;25:506–11.
68. Paoluzi OA, Blanco del VG, Caruso R, Monteleone I, Monteleone G, Pallone F. Impairment of ghrelin synthesis in *Helicobacter pylori*-colonized stomach: new clues for the pathogenesis of *H. pylori*-related gastric inflammation. World J Gastroenterol. 2014;20:639–46.
69. Patel AD, Stanley SA, Murphy KG, Frost GS, Gardiner JV, Kent AS, et al. Ghrelin stimulates

insulin-induced glucose uptake in adipocytes. Regul Pept. 2006;134:17–22.

70. Poykko SM, Kellokoski E, Horkko S, Kauma H, Kesaniemi YA, Ukkola O. Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes. 2003;52:2546–53.
71. Dogan Z, Sarikaya M, Ergul B, Filik L. The effect of *Helicobacter pylori* eradication on insulin resistance and HbA1c level in people with normal glucose levels: a prospective study. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2015;159:242–5.
72. Cheng KP, Yang YJ, Hung HC, Lin CH, Wu CT, Hung MH, et al. *Helicobacter pylori* eradication improves glycemic control in type 2 diabetes patients with asymptomatic active *Helicobacter pylori* infection. J Diabetes Investig. 2019;10:1092–101.
73. Choi YJ, Kim N, Yoon H, Shin CM, Park YS, Park JH, et al. Increase in plasma acyl ghrelin levels is associated with abatement of dyspepsia following *Helicobacter pylori* eradication. J Gastroenterol. 2016;51:548–59.
74. Rask E, Olsson T, Soderberg S, Johnson O, Seckl J, Holst JJ, et al. Impaired incretin response after a mixed meal is associated with insulin resistance in nondiabetic men. Diabetes Care. 2001;24:1640–5.
75. Holst JJ. The physiology of glucagon-like peptide 1. Physiol Rev. 2007;87:1409–39.
76. Zietek T, Rath E. Inflammation meets metabolic disease: gut feeling mediated by GLP-1. Front Immunol. 2016;7:154.
77. Ianiro G, Tilg H, Gasbarrini A. Antibiotics as deep modulators of gut microbiota: between good and evil. Gut. 2016;65:1906–15.
78. Buzas GM. Metabolic consequences of *Helicobacter pylori* infection and eradication. World J Gastroenterol. 2014;20:5226–34.
79. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, et al. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med. 2004;351:2599–610.
80. el-Omar EM, Penman ID, Ardill JE, Chittajallu RS, Howie C, KE MC. *Helicobacter pylori* infection and abnormalities of acid secretion in patients with duodenal ulcer disease. Gastroenterology. 1995;109:681–91.
81. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209–49.
82. Ohtani M, Ge Z, García A, Rogers AB, Muthupalani S, Taylor NS, et al. 17β-Estradiol suppresses *Helicobacter pylori*-induced gastric pathology in male hypergastrinemic INS-GAS mice. Carcinogenesis. 2011;32:1244–50.
83. Yu Y, Cai J, Song Z, Wang J, Wu L. Association of *Helicobacter pylori* infection with metabolic syndrome in aged Chinese females. Exp Ther Med. 2019;17:4403–8.
84. Ohtani M, García A, Rogers AB, Ge Z, Taylor NS, Xu S, et al. Protective role of 17 beta-estradiol against the development of *Helicobacter pylori*-induced gastric cancer in INS-GAS mice. Carcinogenesis. 2007;28:2597–604.

Part IX

The Effect of *H. pylori* on the Gastric Microbiota

59

The Effect of *H. pylori* Infection on the Gastric Microbiota

Nayoung Kim and Hyun Jin Jo

Abstract

Recently, the development of culture-independent molecular technologies such as next-generation sequencing (NGS) has increased an attention to the research about gastric microbiota including *Helicobacter pylori* (*H. pylori*) in the stomach. The composition of gastric microbiota is affected by various factors like a type of samples (gastric juice vs. mucosa), dietary habits, disease status, pH, and *H. pylori* infection. There is a cross-talk between the microbiota and *H. pylori* but a little is known about the role of non-*H. pylori* microbiota in human health and disease. In systemic review regarding the role of non-*H. pylori* bacteria on gastric carcinogenesis, an increase of many bacteria (such as *Lactobacillus coleohominis*, *Klebsiella penumoniae*, or *Acinetobacter baumannii*) as well as decrease in others (*Porphyromanas* spp., *Neisseria* spp., *Prevotella pallens,* or *Steptococcus sinensis*) has been associated with gastric cancer (GC). In general, there is a strong inverse association between *H. pylori* relative abundance and bacterial diversity, but this association becomes weak in GC samples which tended to have lower bacterial diversity. Higher diversity and higher composition of *S. pseudopneumoniae*, *S. parasanguinis*, and *S. oralis* were found in the corpus mucosa of *H. pylori*-negative GC. This suggests that the analysis of microbiota from corpus mucosa could be beneficial to identify a role of non-*H. pylori* bacteria than from antral mucosa. Gastric microbiota other than *H. pylori* are supposed to play a role in the gastric carcinogenesis by the way of microbiota itself or the microbial metabolites/bacterial genotoxins interplay through immune mechanism. Further studies are needed to elucidate the role of non-*H. pylori* microbiota in the gastric carcinogenesis.

Keywords

Helicobacter pylori · Microbiota
Pyrosequencing · Gastric cancer

59.1 Introduction

The discovery of *Helicobacter pylori* (*H. pylori*) in the 1980s brought about a change in the conventional concept that the stomach is a sterile organ. Thereafter, the interest in the microbial

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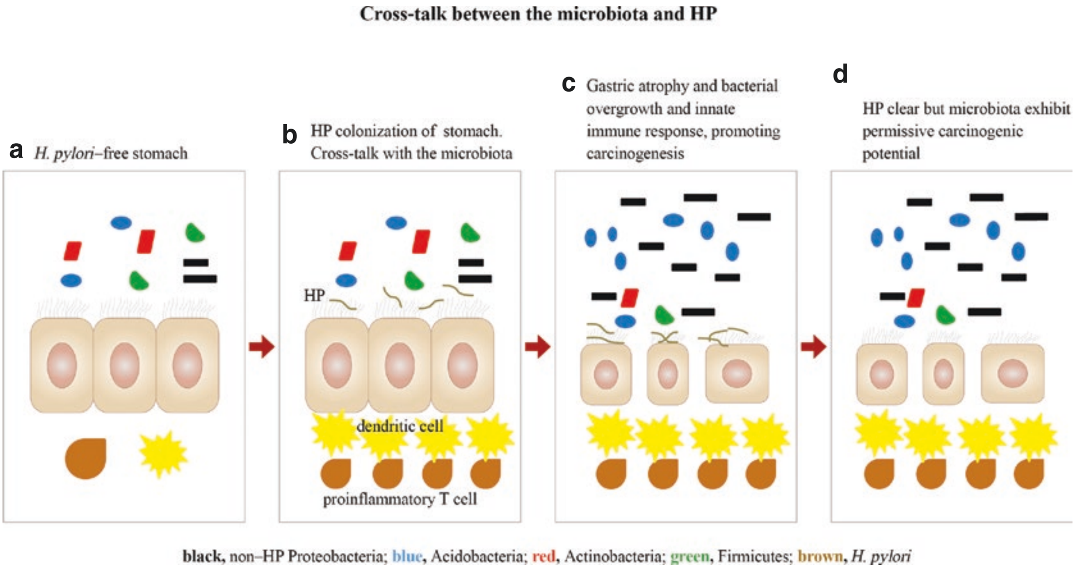

Cross-talk between the microbiota and HP
a H. pylori–free stomach
b HP colonization of stomach. Cross-talk with the microbiota
c Gastric atrophy and bacterial overgrowth and innate immune response, promoting carcinogenesis
d HP clear but microbiota exhibit permissive carcinogenic potential
HP
dendritic cell
proinflammatory T cell
black, non–HP Proteobacteria; blue, Acidobacteria; red, Actinobacteria; green, Firmicutes; brown, H. pylori

Fig. 59.1 Model for cross-talk between the microbiota and *Helicobacter pylori* (*H. pylori*) in predisposing the gastric mucosa for the development of cancer. (**a**) In the *H. pylori*–free stomach, the microbiota coexists peacefully with the gastric mucosa. (**b**) Initial *H. pylori* colonization of the stomach, initiating cross-talk with the microbiota. (**c**) After years of co-colonization, the gastric mucosa atrophies, leading to an increase in pH and changes in nutrient availability and innate immune responses that together promote outgrowth of bacteria that otherwise would not tolerate the gastric environment. *H. pylori* and possibly certain members of the expanded microbiota elicit a strong innate and adaptive inflammatory response that damages the epithelial cell layer, establishing conditions that may promote carcinogenesis. (**d**) After decades of colonization with loss of its ecological niche, *H. pylori* may clear, but the atrophy and nonindigenous microbiota persist, along with their permissive carcinogenic potential (Adapted from Brawner et al. [2])

ecosystem of the stomach has increased, but the actual researches were mainly focused on *H. pylori* [1]. The state of chronic infection of *H. pylori* has proven to cause the inflammation of the gastric mucosa, atrophy, intestinal metaplasia (IM), and further premalignant changes like dysplasia. Thus, it is clear that *H. pylori* plays an important role in the development of gastric cancer (GC). Recently, the development of culture-independent molecular technologies such as next-generation sequencing (NGS) has increased an attention to the research about gastric microbiota. And it was found that most of the gastric microbiota in humans belong to five phyla, including Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Fusobacteria. Model for cross-talk between the microbiota and *H. pylori* shows that there are steps along the chronic gastrtitis and gastric carcinogenesis [2] (Fig. 59.1). In addition, the analysis of gastric microbiota is affected by the location of sampling, dietary habits, disease status, medication like antibiotics or gastric acid secretion inhibitor. In this chapter, the research regarding the gastric microbiota including *H. pylori* will be introduced. In addition, the effect of *H. pylori* infection on the non-*H. pylori* gastric microbiota will be touched.

59.2 The Development of Analysis Methods of Gastric Microbiota

The previous method for the analysis of gastric microbiota was mainly culture-based study using gastric fluid or gastric mucosal tissue. However, in the 1980s, along with the development of molecular biologic analysis technique like DGGE (denaturing gradient gel electrophoresis), T-RFLP (terminal restriction fragment length polymorphism), and microarray, it has been known that a diverse microbiota other than *H. pylori* exists in the stomach [1]. Actually, 80 percent of bacteria known to exist in the stomach were not observed in the culture-based study mainly because the quickly growing microbiota cover the plate; thus, make it difficult to find the colony which grows slowly. In addition, anaerobe cannot grow over the plate in the aerobic condition.

Recently, a new analysis technique has been developed such as NGS, which sequences the genes of bacteria and finds out the species stage. After Bik et al. in the 2006 firstly published the results using 16S ribosomal RNA (rRNA) analysis, recently many studies have been announced about the gastric microbiota [3–6]. Although the NGS molecular approach is powerful and provides a complete view of the overall microbiota, it is unable to distinguish between living and dead bacteria. This is because bacterial genes are present, even if bacteria are killed by antibiotics or cannot survive due to hostile surroundings. However, it is an advantage that NGS shorten the time required for analysis and can analyze simultaneously the large amount of samples [5, 7].

59.3 Various Conditions Affecting the Gastric Microbiota

Stomach plays an important role in maintaining gastrointestinal (GI) health, as a barrier against ingested infectious disease agents of the lower GI tract [8]. In healthy subjects, swallowed pathogens are inactivated by gastric fluid, which contains both hydrochloric acid and proteolytic enzymes [9]. Atrophic gastritis (AG), gastric surgery or drugs that inhibit acid secretion can cause hypochlorhydria [8] and decreased gastric acid secretion is responsible for an increased risk of infection. Thus, gastric microbiota will be affected by acid secretion and by various human host parameters, including immune status, gastric fluid pH, use of proton pump inhibitors (PPIs), and antibiotic medications. In addition, gastric mucosa is divided into antrum and corpus at which acid secretion is very different; thus, there could be much difference of gastric microbiota between antrum and corpus.

59.3.1 The Effect of Sampling Site on the Gastric Microbiota Analysis: Mucosa vs. Gastric Fluid

The most representative phyla of gastric microbiota are Firmicutes, Proteobacteria, Actinobacteria, Fusobacteria, and Bacteroidetes [10] (Fig. 59.2). The composition of Firmicutes and Proteobacteria is major in the gastric mucosa, but, in the gastric fluid, Actinobacteria, Bacteroidetes, and Firmicutes relatively accounted more proportion [3]. *H. pylori* belonging to the Proteobacteria is found less in the gastric fluid. Actually, in the pyrosequencing study of our laboratory, *H. pylori* was accounted an average of 66.5% in the gastric mucosa, but only 3.3% in gastric fluid [11] (Fig. 59.3). This is because a suitable place that *H. pylori* live together is not the gastric fluid but gastric epithelial cells and the mucus adjacent to the mucosa [12].

Contrary to the previous studies about stomach environment based on biopsy samples from the mucosa [3, 4], our study showed that *H. pylori* was not the dominant species in the gastric fluid microbiota [11]. This result is similar to other reports [13, 14], confirming that *H. pylori* colonizes the mucous layer and adheres to gastric epithelial cells [12]. In addition, the ratio of culture recovery from gastric fluid (5/12) was about half compared to that from gastric mucosa (10/12) [5].

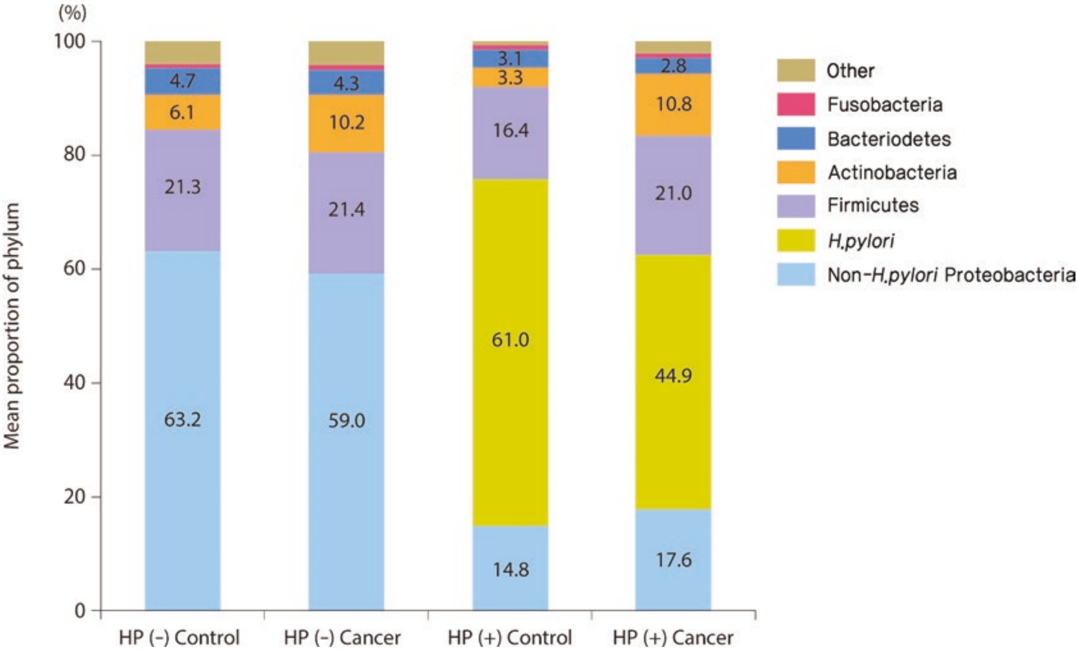


Fig. 59.2 The mean proportion of phylum. The most representative phylum was Proteobacteria, followed by Firmicutes, Actinobacteria, Bacteroidetes, and Fusobacteria. *HP H. pylori* (Adapted from Jo et al. [10])

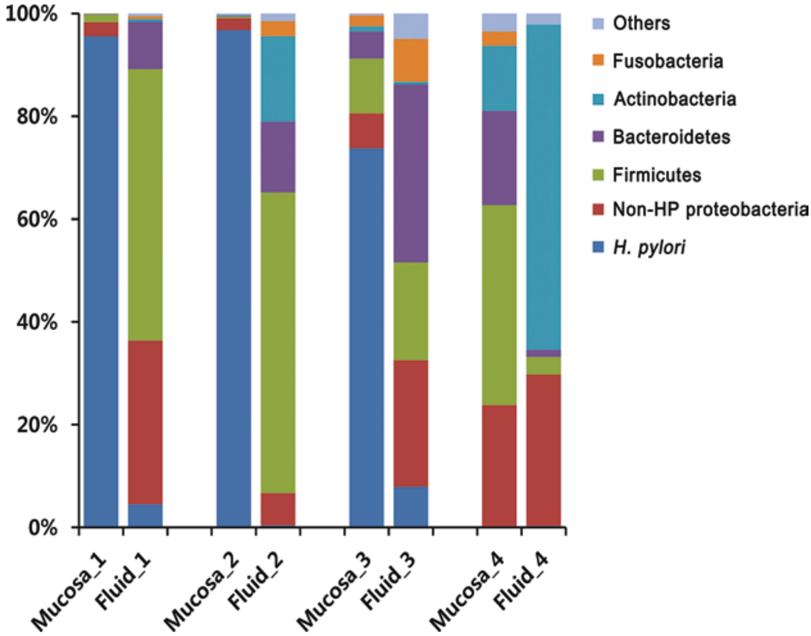


Fig. 59.3 The composition of phylum in the gastric mucosa and gastric fluid samples. The phylum composition was different between gastric mucosa samples and gastric fluid samples. The proportion of *H. pylori* was higher in the gastric mucosa samples (66.5%) compared with that in gastric fluid samples (3.3%). *HP H. pylori* (Adapted from Sung et al. [11])

59.3.2 Corpus Biopsy Was More Favorable for the Analysis Regarding a Possible Role of Bacteria Other than *H. pylori* in the Gastric Carcinogenesis

Since the parietal cells that secrete gastric acid are mainly distributed in the gastric corpus than in the antrum, the acidity of the gastric corpus is normally higher than that of the antrum. Consequently, the antrum and corpus of stomach are distinct niches for microbial colonization owing to differential ability to secrete gastric acid [15]. In the previous studies, there was no significant difference of gastric microbiota depending on the site of the harvested tissue [14, 16]. However, it can be different when the gastric condition changes to AG or IM [17].

Initial study using 17 corpus-antrum paired samples did not show the significant differences of gastric microbiota between antrum and corpus [10]. However, when 12 subjects (two *H. pylori*-negative controls, two *H. pylori*-negative cancer, three *H. pylori*-positive controls, and five *H. pylori*-positive cancer patients) were analyzed again to evaluate the role of non-*H. pylori* bacteria in the gastric carcinogenesis that there was a difference of non-*H. pylori* bacteria between antrum and corpus [17]. For instance, the means of reads and operational taxonomic units (OTUs) were lower in gastric antral mucosa samples (Fig. 59.4a) than gastric corpus mucosa samples [17] (Fig. 59.4b). Gastric corpus mucosa samples showed higher diversity compared to antrum mucosa samples [17] (Fig. 59.4a and 4b). The unweighted UniFrac analysis indicated that there was very little separation between control and cancer groups but the separation became clear depending on *H. pylori* infection status in gastric antral mucosa [10] (Fig. 59.5).

However, *H. pylori*-negative groups showed a separation between the control and cancer group in both of antrum and corpus [17]. The bacterial communities at the phylum level among four groups showed that the proportion of Proteobacteria of the *H. pylori*-positive groups was greater than that of the *H. pylori*-negative cancer group [17]. In the *H. pylori*-negative control group, *Pseudomonas hibiscicola* and *Ralstonia pickettii* were more abundant in antrum (44.87% vs. 0%) than in corpus mucosa (45.90% vs.0.03%) [17] (Table 59.1). In the *H. pylori*-negative control group, *Bradyrhizobium* sp. (*B. jicamae*, *B. pachyrhizi*, *B. denitrificans*, and *B.* g1 uc) were more abundant in corpus (8.66%, 5.33%, 1.14%, and 0.72%, respectively) than in antrum mucosa (all 0%, Table 59.1). Actinobacteria of the *H. pylori*-positive cancer group was greater than that of the *H. pylori*-negative cancer group. However, *Propionibacterium acnes* was more abundant in corpus than antrum mucosa (8.49% vs. 1.43%, Table 59.1) [17]. The proportion of Firmicutes in the *H. pylori*-negative groups was greater than the *H. pylori*-positive groups. These differences were more pronounced in the antral mucosa [17]. The bacterial communities at the species level among four groups in gastric antral and corpus mucosa showed that the proportion of *Streptococcus* sp. in the *H. pylori*-negative cancer group was greater than the others (Table 59.1). The proportion of *S. mitis* group [18–20], such as *S. pseudopneumoniae, S. mitis, S. infantis, S. oralis*, and *S. tigurinus*, in the *H. pylori*-negative cancer group was higher than those in the *H. pylori*-positive cancer group (antrum, 33.9 vs. 8.4, $p = 0.076$; corpus, 34.1 vs. 0.33, $p = 0.009$, Table 59.1). Statistical analysis showed that there was correlation between *H. pylori*-negative cancer and *S. mitis* group, especially in the corpus mucosa.

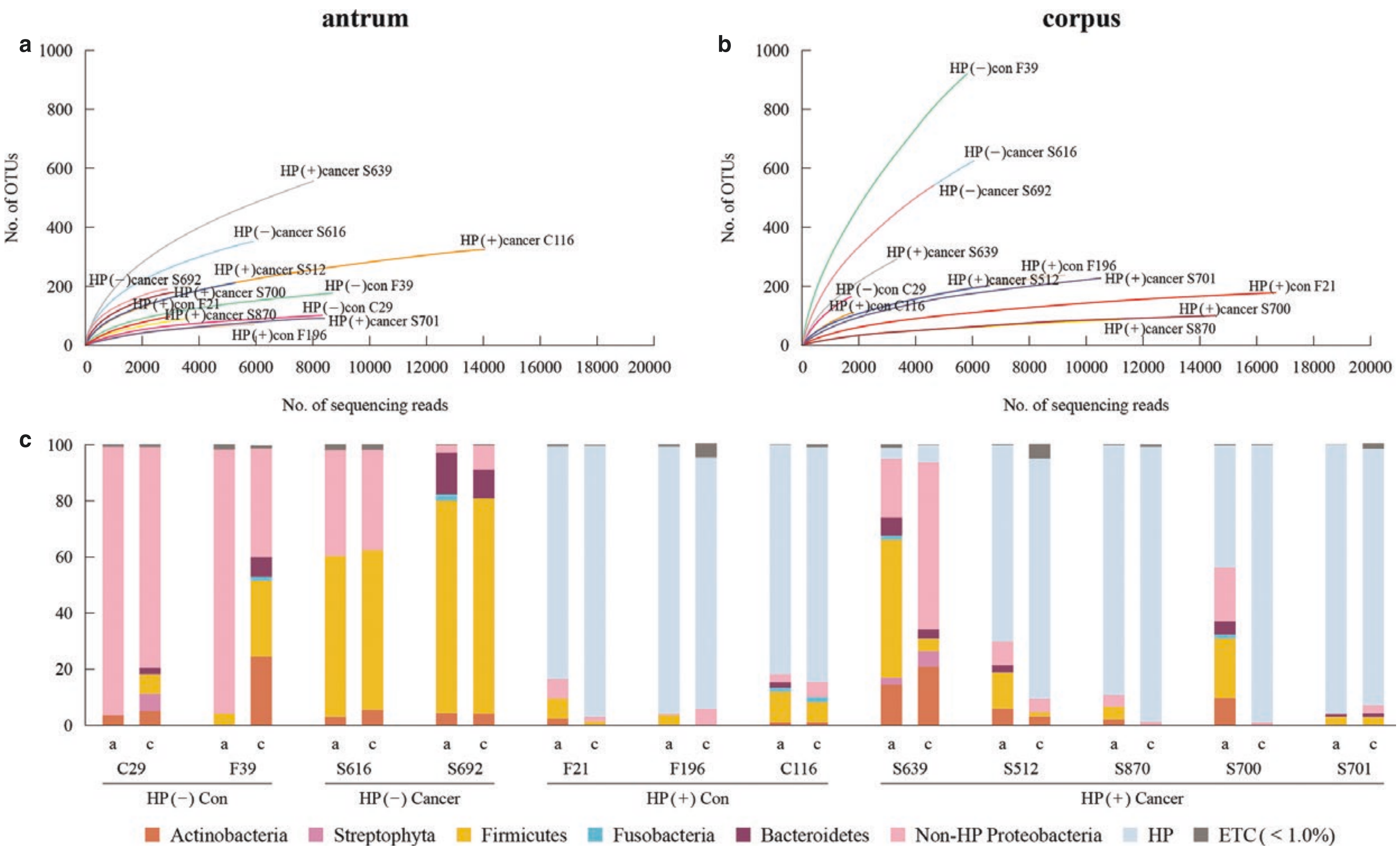


Fig. 59.4 Bacterial diversity in gastric antrum and corpus mucosa samples. (**a**) Refraction curves indicating the number of assigned bacterial genera in relation to the number of 16S rRNA sequences in the antrum. (**b**) In general, OTU number in the corpus is higher than that of antrum. (**c**) Taxonomic assignment of the 24 samples at the level of bacterial phylum. *HP H. pylori*, *OTU* operational taxonomic units (Adapted from Sohn et al. [17])

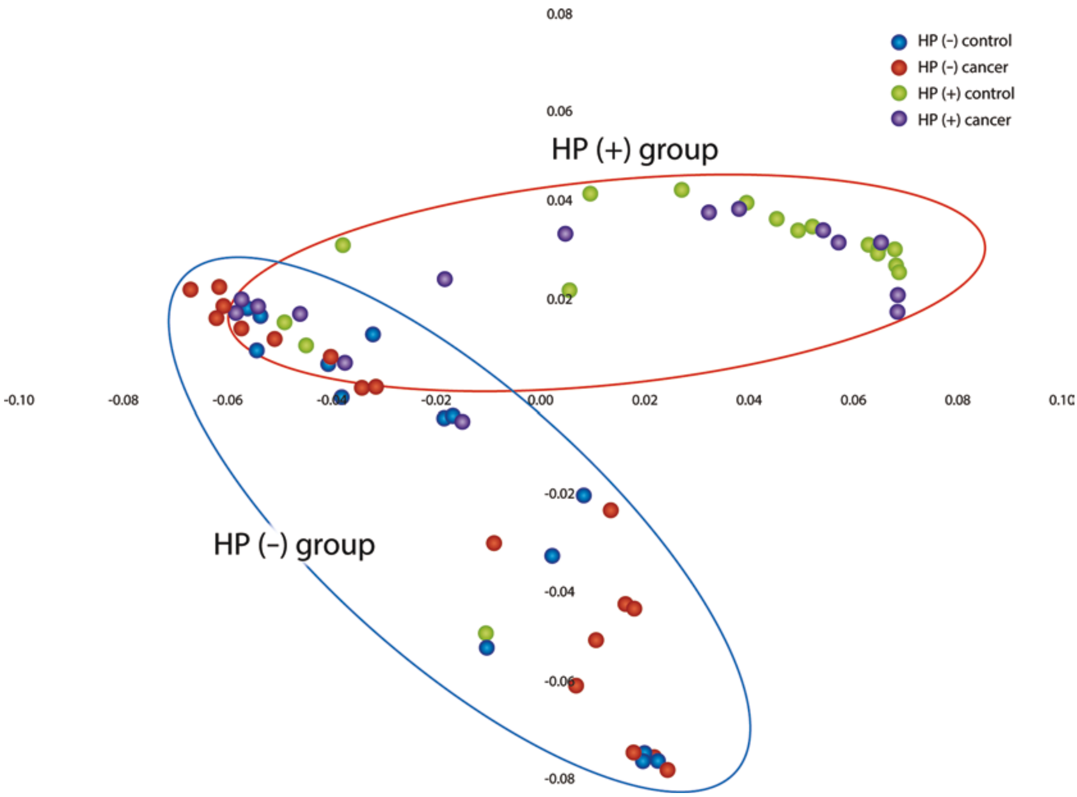


Fig. 59.5 Unweighted UniFrac-based principal coordinates analysis of gastric antral microbial communities. This analysis is based on taxa clustered at 97% sequence 16S rRNA gene identity. The unweighted UniFrac analysis indicated that there was very little separation between control and cancer groups under the identical *H. pylori* infection status. Samples obtained from patients of *H. pylori*-negative control, *H. pylori*-negative cancer, *H. pylori*-positive control, and *H. pylori*-positive cancer are represented by *blue*, *red*, *green*, and *purple circles*, respectively. *HP H. pylori* (Adapted from Jo et al. [10])

59.3.3 Influence of Change of pH on the Gastric Microbiota

It has been known that increased gastric pH by the long-term use of PPIs or H_2 receptor blocker bring the bacterial overgrowth and the change of composition of gastric microbiota. These changes were prominent under the longer administration of acid lowering drug and the infected state with *H. pylori*. And the bacterial overgrowth consists mainly of oral bacteria [21]. In the condition of achlorhydria related with aplastic anemia, coliform was found in the stomach [22].

Table 59.1 Comparison of species frequency of gastric antrum and corpus mucosal samples in the control and gastric cancer (Adapted from Sohn et al. [17])

		Antrum (%)				Corpus (%)			
Phylum	Species	HP (−) control ($n = 2$)	HP (−) Cancer ($n = 2$)	HP (+) control ($n = 3$)	HP (+) Cancer ($n = 5$)	HP (−) control ($n = 2$)	HP (−) Cancer ($n = 2$)	HP (+) control ($n = 3$)	HP (+) Cancer ($n = 5$)
Firmicutes	*Streptococcus pseudopneumoniae*	0.14	**18.68**	**3.37**	**6.04**	**3.80**	**23.11**	0.98	0.20
	S. mitis	0.02	**9.01**	0.51	0.82	0.45	**3.09**	0.23	0.00
	S. salivarius	0.01	**4.00**	0.11	0.40	0.27	**6.37**	0.03	0.02
	S. infantis	0.32	**3.39**	0.24	**1.10**	**3.07**	**5.38**	0.08	0.12
	Veillonella atypica	0.03	**2.97**	0.23	0.64	0.32	**1.22**	0.03	0.01
	V. dispar	0.03	**2.37**	0.18	0.77	0.53	**1.25**	0.05	0.01
	Granulicatella adiacens	0.04	**3.33**	0.08	0.71	0.51	**1.98**	0.03	0.02
	Gemella haemolysans	0.00	**2.09**	0.19	0.20	0.03	**1.16**	0.05	0.01
	S. australis	0.05	**2.19**	0.14	0.68	0.59	**2.01**	0.10	0.01
	S. parasanguinis	0.14	**1.51**	0.27	0.60	0.86	**3.36**	0.14	0.04
	S._uc	0.10	**1.14**	0.20	0.65	0.76	**2.96**	0.06	0.06
	GQ130066_s	0.01	0.90	0.21	0.54	0.00	0.00	0.00	0.00
	S. oralis	0.02	**1.72**	0.14	0.30	0.50	**2.07**	0.08	0.01
	Lactobacillus salivarius	0.00	**1.93**	0.00	0.05	0.01	0.67	0.00	0.00
	S. tigurinus	0.01	**1.06**	0.03	0.17	0.25	0.43	0.00	0.00
	Solobacterium moorei	0.02	0.17	0.03	0.54	0.01	0.13	0.00	0.02
	S. lactarius	0.02	0.88	0.05	0.08	0.14	0.65	0.02	0.00
	Megasphaera micronuciformis	0.00	0.70	0.02	0.15	0.03	0.04	0.00	0.00
	Streptococcaceae_uc_s	0.02	0.41	0.10	0.11	0.57	**1.90**	0.12	0.05
	Lactobacillales_uc_s	0.01	0.19	0.02	0.08	0.82	**2.09**	0.07	0.05

Proteobacteria	*Helicobacter pylori*	0.11	0.11	**86.47**	**60.30**	0.42	0.10	**89.78**	**75.93**
	Haemophilus parainfluenzae	0.15	**3.84**	0.46	0.96	**2.15**	**2.41**	0.13	0.02
	Escherichia coli group	0.03	0.00	0.00	**1.46**	0.00	0.01	0.00	0.05
	H. paraphrohaemolyticus	0.00	**1.60**	0.08	0.60	0.03	**1.10**	0.02	0.01
	Methylobacterium adhaesivum	0.06	**2.22**	0.12	0.56	0.06	0.00	0.00	0.05
	Bradyrhizobium jicamae	0.00	0.07	0.01	0.22	**8.66**	0.68	0.24	**1.24**
	Neisseria perflava	0.00	**4.24**	**1.14**	0.17	0.04	**5.12**	0.04	0.02
	Pseudomonas beteli	**1.05**	0.05	0.01	0.20	0.00	0.00	0.00	0.00
	P. hibiscicola	**44.87**	0.06	0.24	0.17	0.00	0.02	0.00	0.01
	Helicobacteraceae_uc_s	0.01	0.00	0.08	0.07	0.06	0.03	**1.28**	0.49
	H._uc	0.00	**1.00**	0.07	0.11	0.09	0.86	0.04	0.00
	Aggregatibacter segnis	0.02	**1.07**	0.00	0.05	0.00	0.44	0.00	0.01
	H. haemolyticus	0.00	0.61	0.10	0.04	0.04	0.53	0.04	0.00
	Ralstonia pickettii	**45.90**	0.05	0.02	0.04	0.03	0.00	0.00	0.00
	Pasteurellaceae_uc_s	0.01	0.19	0.02	0.03	0.36	0.69	0.02	0.01
	Pasteurellales_uc_s	0.00	0.04	0.01	0.02	0.12	0.63	0.00	0.01
	B. pachyrhizi	0.00	0.01	0.00	0.03	**5.33**	0.49	0.11	0.70
	Klebsiella pneumoniae	0.00	0.07	0.00	**1.05**	0.00	0.02	0.00	0.03
	Neisseriaceae_uc_s	0.00	0.22	0.00	0.02	0.05	0.60	0.00	0.00
	Neisseriales_uc_s	0.01	0.05	0.01	0.01	0.08	0.80	0.00	0.00
	FJ269053_s	0.02	0.08	0.00	0.01	**14.23**	**1.38**	0.46	**2.94**
	B. denitrificans	0.00	0.07	0.00	0.00	**1.14**	0.09	0.03	0.10
	U87765_s	0.02	0.02	0.00	0.00	**2.97**	0.27	0.08	0.51
	Bradyrhizobiaceae_uc_s	0.00	0.00	0.00	0.01	0.98	0.20	0.04	0.13
	DQ532251_g_uc	0.00	0.00	0.00	0.00	0.79	0.09	0.03	0.18
	Pelomonas saccharophila	0.00	0.00	0.00	0.00	**3.26**	0.47	0.22	0.95
	Rhizobium hainanense	0.00	0.00	0.00	0.00	**1.10**	0.11	0.04	0.24
	M. longum	0.00	0.00	0.00	0.00	**1.91**	0.09	0.06	0.40
	M. radiotolerans	0.01	0.00	0.00	0.00	**3.08**	0.18	0.11	0.62
	B._g1_uc	0.00	0.00	0.00	0.00	0.72	0.09	0.02	0.09

(continued)

Table 59.1 (continued)

Phylum	Species	Antrum (%)				Corpus (%)			
		HP (−) control ($n = 2$)	HP (−) Cancer ($n = 2$)	HP (+) control ($n = 3$)	HP (+) Cancer ($n = 5$)	HP (−) control ($n = 2$)	HP (−) Cancer ($n = 2$)	HP (+) control ($n = 3$)	HP (+) Cancer ($n = 5$)
Bacteroidetes	*Prevotella histicola*	0.01	**3.08**	0.11	0.43	0.11	**1.85**	0.08	0.00
	P. melaninogenica	0.01	**1.56**	0.06	0.84	0.14	0.92	0.04	0.02
	P._uc	0.01	**1.07**	0.23	0.31	0.21	0.98	0.06	0.01
	P. pallens	0.02	0.65	0.08	0.33	0.03	0.31	0.00	0.02
	P. salivae	0.00	0.78	0.06	0.14	0.05	0.46	0.02	0.01
	EF123551_g_uc	0.00	0.00	0.00	0.00	**1.45**	0.11	0.07	0.51
Actinobacteria	*Propionibacterium acnes*	**1.43**	0.42	0.82	**4.42**	**8.49**	**1.39**	0.27	**4.34**
	Actinomyces odontolyticus	0.05	**1.31**	0.17	0.89	0.44	0.95	0.12	0.03
	Rothia mucilaginosa	0.09	0.26	0.07	0.12	0.90	0.09	0.05	0.01
	Propionibacterium_uc	0.02	0.01	0.01	0.04	0.53	0.07	0.01	0.07
	Propionibacteriaceae_uc_s	0.04	0.04	0.00	0.02	0.79	0.18	0.04	0.16
Viridiplantae	*Prunus persica*	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.83
	Nicotiana tabacum	0.00	0.00	0.00	0.00	**1.09**	0.00	0.00	0.05
	Ipomoea purpurea	0.00	0.00	0.00	0.00	**1.59**	0.00	0.00	0.00
Fusobacteria	*Fusobacterium nucleatum*	0.09	0.92	0.26	0.11	0.33	0.16	0.08	0.03

Values are expressed as mean percent. Bold style means cutoff >1.0

59.4 The Effect of *H. pylori* Infection on the Gastric Microbiota

Model for cross-talk between the microbiota and *H. pylori* showed that there is a serial of changes of relationship between the microbiota and *H. pylori* along the gastric carcinogenesis [2] (Fig. 59.1). For instance in the *H. pylori*–free stomach, the microbiota coexists peacefully with the gastric mucosa (Fig. 59.1a) and initial *H. pylori* colonization of the stomach initiats cross-talk with the microbiota (Fig. 59.1b). After years of co-colonization, the gastric mucosa atrophies, leading to an increase in pH and changes in nutrient availability and innate immune responses that together promote outgrowth of bacteria that otherwise would not tolerate the gastric environment (Fig. 59.1c). *H. pylori* and possibly certain members of the expanded microbiota elicit a strong innate and adaptive inflammatory response that damages the epithelial cell layer, establishing conditions that may promote carcinogenesis [2]. After decades of colonization with loss of its ecological niche, *H. pylori* is kicked off, but the atrophy and nonindigenous microbiota persist (Fig. 59.1d), along with their permissive carcinogenic potential [2].

59.4.1 Under the Normal Acidic Condition of a Healthy Stomach Without *H. pylori*

Under the normal acidic condition of a healthy stomach, which was not infected with *H. pylori*, *Veillonella* spp., *Lactobacillus* spp., *Clostridium* spp., and *Corynebacterium* spp. were mainly cultured [23]. In addition, based on an arbitrary cut-off value requiring genera to be present in more than 20% of the included studies, the typical gastric microbiota consists of 57 bacterial genera distributed among eight phyla, including Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria, Spirochetes, Tenericutes, and TM7 [24]. The six most common genera reported were *Prevotella, Streptococcus, Neisseria, Hemophilus, Fusobacterium*, and *Veillonella* [25]. Another study showed similar results that *Streptococcus*, *Prevotella*, *Neisseria*, *Haemophilus*, and *Porphyromonas* genera accounted for about 71% of gastric microbiota. Especially, *Streptococcus* and *Prevotella* genera were equivalent to 41% of the total bacteria [5]. Thus, *Streptococcus* is a bacterium that is most commonly found in healthy stomach. *Streptococcus mitis* is known as aciduric bacterium that can propagate under the acidic condition [22]. When *H. pylori* enters into stomach, gastric microbial communities become very different according to *H. pylori* infection or not [10] (Fig. 59.5). However, the unweighted UniFrac analysis indicated that there was very little separation between control and cancer groups under the identical *H. pylori* infection status [10] (Fig. 59.5).

59.4.2 What Happens When *H. pylori* Infection Occurs

Most reports show that *H. pylori*–positive and *H. pylori*–negative individuals' microbiota are mainly dominated by the same phyla but with different percentages of relative abundance [3, 26, 27]. *H. pylori*–positive individuals have a higher abundance of Proteobacteria, probably resulting from the contribution of *H. pylori*, while there is a lower abundance of Actinobacteria, Bacteroidetes, and Firmicutes [26–28].

As soon as *H. pylori* infection occurs, *H. pylori* occupies the most of gastric microbiota. Therefore, microbial diversity is reduced under the *H. pylori*-infected stomach compared to healthy stomach [3, 4]. However, in the state of chronic infection of *H. pylori*, gastric microbial diversity increases again. There are possible explanations for this. The first, *H. pylori*-induced inflammation causes gastric atrophy and reduces the parietal cell mass, elevating gastric pH, which predisposes to colonization by environmental microbiota that cannot survive in the normal gastric pH [29]. There are other plausible reasons for *H. pylori*-induced changes in the microflora. *H. pylori* produces ammonia and bicarbonate from urea that can be used as substrates by other bacteria [30], and *H. pylori* infection is correlated with slower migrating motor complex (MMC) phase III activity, which contributes to the less clearing of adherent bacteria from the antral mucosal compartment [14].

Even though there had been reports that chronic *H. pylori* infection did not alter the gastric microbiota [3, 31], recent study showed that the diversity of gastric microbiota increased with progression from chronic gastritis and IM to GC, and this change was prominent in *H. pylori*-positive group [6]. However, it is different in the rhesus macaque model [32]. That is, when Martin et al. assessed the impact of *H. pylori* on the pre-existing gastric microbial community [32], there was no significant difference in the average relative abundance of non-*Helicobacter* taxa in pre- and post-inoculation samples after removing *Helicobacter* reads in the rhesus macaque model [32]. This rhesus model suggests the rhesus gastric microbial community is largely stable despite the immunological and physiological changes that occur due to *H. pylori* infection, which is quite different from human stomach.

59.4.3 An Appropriate Cutoff Value for Determining the Colonization of *Helicobacter pylori* by the Pyrosequencing Method

Sequencing of 16S rRNA gene has improved the characterization of microbial communities. It enabled the detection of low abundance gastric *H. pylori* sequences even in subjects that were found to be *H. pylori*-negative with conventional methods. Thus, a reasonable cutoff value for *H. pylori*-colonization in gastric mucosa samples by the pyrosequencing method is necessary. To evaluate this issue, we performed a study regarding this issue by 16S rDNA pyrosequencing [33]. Gastric mucosal biopsies were taken from 63 subjects whose *H. pylori* status was determined by a combination of serology, rapid urease test, culture, and histology [33]. Of the 63 subjects, the presence of *H. pylori* sequences was detected in all (28/28) conventionally *H. pylori*-positive samples and in 60% (21/35) of *H. pylori*-negative samples. The average percent of *H. pylori* reads in each sample was 0.67 ± 1.09% in the *H. pylori*-negative group. The cutoff value for clinically positive *H. pylori* status was approximately 1.22% based on the ROC-curve analysis (AUC [area under the curve] 0.957; $p < 0.001$). Our results suggest that the cutoff value of *H. pylori* sequence percentage for the *H. pylori* colonization by pyrosequencing could be set at approximately 1%. As there was no study regarding this issue, this cutoff value for *H. pylori*-colonization in gastric mucosa might be helpful to analyze gastric microbiota related to *H. pylori* sequence status [33].

59.5 Altered Microbiota Composition Related with Disease State

Eun et al. [6] showed that, under the *H. pylori*-positive state, the GC group showed a significant decrease in the Epsilon proteobacteria class and Helicobacteraceae family compared with the chronic gastritis and IM group, while the Bacilli class and Streptococcaceae family increased significantly [6]. In GC group, the number of *Bifidobacteria*/*Lactobacilli*, *Veillonella*, and *Streptococci* increased as determined by culture [22]. The previous study for the microbial composition of human stomach by Dicksved et al. [34] using T-RFLP with the bacterial sequencing method demonstrated that there was no significant difference in microbial composition of phylum between patients with GC and controls, while genera *Streptococcus*, *Lactobacillus*, *Veillonella*, and *Prevotella* were predominant in patients with GC. Among the *Streptococcus,* a group including *S. mitis* and *S. parasanguinis* was shown to dominate [34]. In addition, Li et al. [16] showed that, even in the case of *H. pylori*-negative patients, *Streptococcus* was significantly higher in the gastritis group compared to the control group. As *Streptococcus* was cultured in a tissue sample subjected to rigorous washing steps, *Streptococcus* is likely to act as pathogen [16].

59.5.1 Altered Microbiota Composition in the Gastric Cancer in the Presence of *H. pylori*

H. pylori infection has been linked to altered GI microbiota and dysbiosis [35, 36]. However, it is not clear whether infection with *H. pylori* itself approves the growth of unwanted microorganisms or an altered microbiota brings about beneficial situations for the colonization of *H. pylori* [37]. Probably, there is a multifaceted interaction, where the *H. pylori* colonization contributes to the growth of some microbes and vice versa [25]. It is likely that dysbiosis alters gastric mucosa which is highly desired for the colonization of *H. pylori* [38]. Some researchers believe that *H. pylori* is more of a latent or opportunistic pathogen than a pathogenic bacterium and can be considered a commensal organism. This is important because we know that the majority of the world's population is infected with *H. pylori* and colonization occurs with bacteria that carry or do not carry critical virulence factors at an early age. However, it should be noted that severe GI diseases or complications occur mainly in adults with age > 40 years and only in <10% of infected individuals. This low incidence clearly suggest that *H. pylori* is more of a latent or opportunistic pathogen than a pathogenic bacterium, and that virulence factors play little role in the outcome of the disease [39]. Long-term colonization of *H. pylori* and its interaction with other gastric microbiota appear to alter gastric mucosal dysbiosis and lead to the development of severe GI disease, including GC, by inducing persistent and long-term inflammatory responses [38, 40]. It can be speculated that the alterations of gut microbiota induced by *H. pylori* may affect the development of GC since the composition of microbiota stimulates immune responses at a systemic and local level; moreover, the development of GC is affected by inflammatory signaling [40]. However, the interaction between gut microbiota and *H. pylori* is not well known yet and the literature reveals inconsistent results [41]. The gastritis activity is perceived for its tight correlation with *H. pylori* infection, which is further approved by similar changes observed in the fecal microbiota from the subjects with non-active gastritis and past infection. Additionally, the same alteration tendencies were found for major genera or phyla, including reduced Bacteroidetes abundance and increased Proteobacteria or Firmicutes abundances, with gastric lesion severity and *H. pylori* infection status (particularly the status of past infection). Furthermore, it states that changes in gut microbiota may develop precancerous gastric lesions related to *H. pylori* and carcinogenesis [40]. The elevation of pH dramatically influences the bacterial growth. The mechanisms by which *H. pylori* and its associated microbiota promotes the atrophy that leads to cancer is unclear. However, there is a possible mechanisms of interaction between *H. pylori* and gastric microbiota to induce gastric carcinogenesis [42] (Fig. 59.6).

One hypothesis is that DNA-damaging reactive oxygen species and reactive nitrogen species produced by the bacterial communities contributes to carcinogenesis [43]. In addition, it has been suggested that lactic acid producing bacteria may promote gastric inflammatory reactions induced by *H. pylori* [44]. Lactic acid bacteria promote immune tolerance, providing the platform for colonization of other carcinogenic bacteria [45]. By modulating the acidity of the stomach, *H. pylori* could change the gastric microbiome profiles, promoting *H. pylori*-associated disorders. Alterations in the gastric environment that decline acid secretion can encourage the growth of *N*-nitroso compounds (NOC)-producing bacteria, thus elevating the chance of gastric carcinogenesis [46]. In addition, Th1/Th17 cells contribute to the inflammatory response of *H. pylori*-infected patients [47, 48]. Inflammation increases gastric pH, which decreases *H. pylori* levels and increases non-*H. pylori* bacteria in the stomach [49]. There is a significant difference in the microbial profiles and composition of early and advanced GC, reflecting the changes related to GC progression. The gastric microbiome alterations in EGC stages could be assigned to host genetic changes, *H. pylori* infection, bacterial virulence, and adapta-

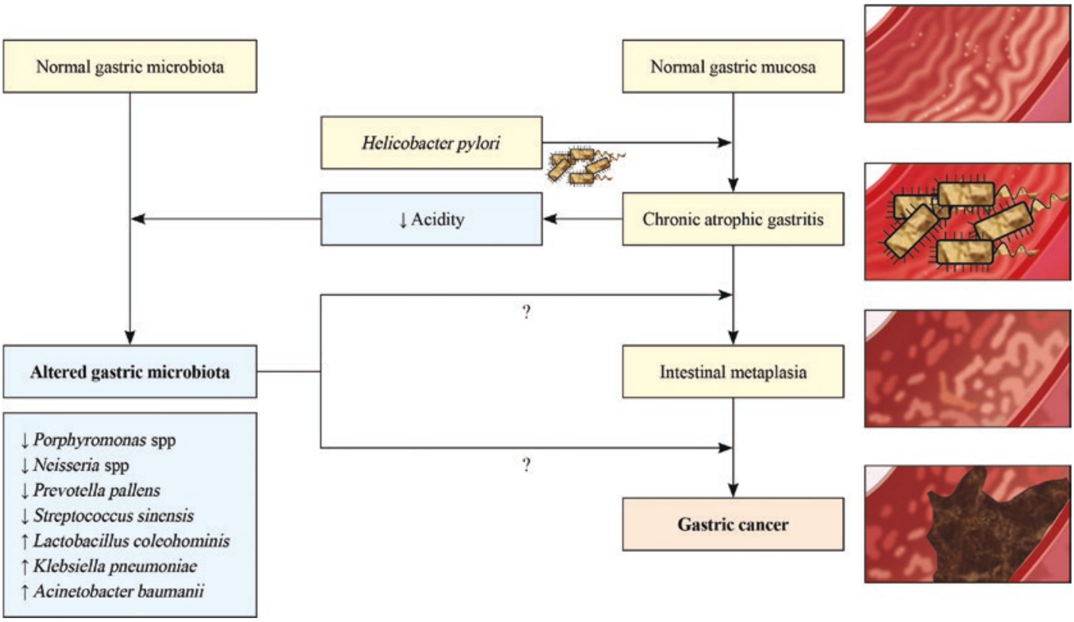

Normal gastric microbiota
Normal gastric mucosa
Helicobacter pylori
↓ Acidity
Chronic atrophic gastritis
?
Altered gastric microbiota
Intestinal metaplasia
↓ *Porphyromonas* spp
↓ *Neisseria* spp
↓ *Prevotella pallens*
↓ *Streptococcus sinensis*
↑ *Lactobacillus coleohominis*
↑ *Klebsiella pneumoniae*
↑ *Acinetobacter baumanii*
?
Gastric cancer

Fig. 59.6 Possible mechanisms of interaction between *Helicobacter pylori* and gastric microbiota to induce gastric carcinogenesis (Adapted from Dias-Jácome et al. [42])

tion to the environment. Constrained principal coordinate analyzes indicated the influence of *H. pylori* and *cagA* and *vacA* genotypes on the gastric microbiome structure. Thus, the detected microbial fingerprint can be regarded as a biomarker for clinical evaluation of GC risk among high-risk cases [50].

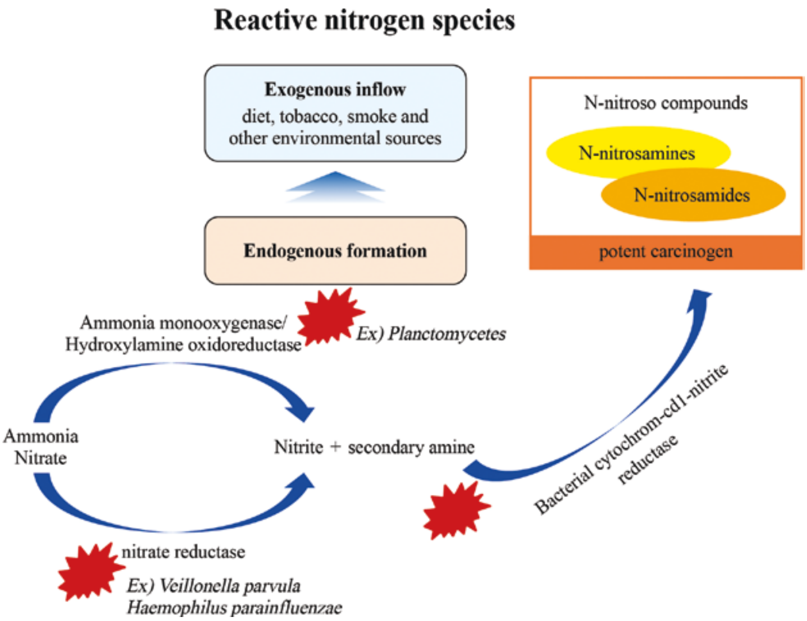
59.5.2 Altered Microbiota Composition in the Gastric Cancer in the Absence of *H. pylori*

The underlying mechanism of the development of GC in the absence of *H. pylori* is nearly not known. However, experts suppose that the gut microbiota other than *H. pylori* might play a role in the gastric carcinogenesis in this condition. Of course, gastric acidity is a barrier to bacterial overgrowth [8, 51] but decreased gastric acid secretion is responsible for an increased risk of infection [18]. For instance, the bacterial colonization in stomach increases under the condition like as acid-reducing drug, AG, nutritional deficiency, and gastric surgery [8, 52]. Li et al. [16] identified that alpha-hemolytic potential pathogens (*S. pneumonia, S. mitis,* and *S. salivarius*) were high in antral gastritis stomach. Similarly, our results also showed that the *H. pylori*–negative cancer group showed high proportion of *Streptococcus* (phylum Firmicutes) in both gastric antrum (41.3%) and corpus (49.5%). However, the proportion of *Streptococcus* sp. was more pronounced in the corpus, suggesting that altered microbiota composition in the corpus could be more important in the development of GC [17].

59.6 Link Between Gastric Microbiota and Gastric Cancer

Many researchers tried to solve the link between the gastric microbiota and GC. There are many components which make the healthy stomach to become tumor microenvironment in which GC easily develops. The microbiota itself or the microbial metabolites/bacterial genotoxins interplay and cooperate in the gastric carcinogenesis by way of immune mechanism [25]. Actually

Fig. 59.7 Exogenous inflow and endogenous formation of reactive nitrogen species and conversion of nitrate into nitrite by bacterial enzymes


Reactive nitrogen species
Exogenous inflow
diet, tobacco, smoke and other environmental sources
Endogenous formation
N-nitroso compounds
N-nitrosamines
N-nitrosamides
potent carcinogen
Ammonia monooxygenase/ Hydroxylamine oxidoreductase
Ex) Planctomycetes
Ammonia
Nitrate
Nitrite + secondary amine
Bacterial cytochrom-cd1-nitrite reductase
nitrate reductase
Ex) Veillonella parvula
Haemophilus parainfluenzae

chronic *H. pylori* infection leads to a decrease in gastric acid secretion, which allows successful colonisation by new bacteria species that may contribute to gastric carcinogenesis by causing persistent inflammation and conversion of nitrates into *N*-nitrosamines [3, 4]. In GC patients, the concentration of nitrite in gastric juice may increase up to 107.6 μmol/L [53]. When the acid output reduces, bacterial overgrowth occurs in the stomach. These bacteria contain both nitrate reductase and nitrite reductase, which catalyze the reduction of nitrate and nitrite, respectively (Fig. 59.7). However, some bacteria have a differential rate in nitrate reduction and nitrite reduction. *Veillonella parvula* and *H. parainfluenzae* have a higher capacity in nitrate reduction than nitrite reduction, thus increasing nitrite accumulation in the gastric juice [53].

The reduction of gastric acidity by antisecretory agents also results in substantial intragastric bacterial overgrowth, increased counts of nitrate-reducing bacteria, and increased nitrite and *N*-nitrosamine levels [54, 55]. This has been confirmed by studies using the hypergastrinemic insulin-gastrin (INS-GAS) transgenic mouse model, which showed that *H. pylori*-induced gastric carcinogenesis was facilitated by the presence of a complex gastric microbiota, with the mice developing more tumours than germ-free mice infected with *H. pylori* only [56, 57].

59.6.1 Nitrosating and/or Urease Producing Bacteria

H. pylori causes AG modulating inflammatory responses and making NOC [52, 58]. NOC can be formed from nitrite and secondary amines by nitrosating bacteria of stomach such as *Clostridium, Veillonella, Haemophilus, Staphylococcus, Streptococcus*, and *Neisseria* [10, 59] as well as *H. pylori*. NOC formation has been suggested to increase the risk of GC [10]. In addition, urease is a major inducer of innate immune response [60–62]. Urease-producing non-*H. pylori* microbes include *Actinomyces, Clostridium, Corynebacterium*, member of the Enterobacteriaceae (*Citrobacter, Enterobacter, Klebsiella, Morganella, Providencia,* and *Proteus*), *Enterococcus*, *Gardnerella*, *Haemophilus, Lactococcus, Mycobacterium, Streptococcus, Staphylococcus, Ureaplasma,* and *Yersinia* were detected in the oral cavity, GI tract, urethrogenital tract, and skin [63–65]. Gastric pH

Table 59.2 Comparison of urease and N-nitroso compounds-producing bacteria between antrum and corpus (Adapted from Sohn et al. [17])

Species	Antrum (%)				Corpus (%)			
	HP (–) control ($n = 2$)	HP (–) Cancer ($n = 2$)	HP (+) control ($n = 3$)	HP (+) Cancer ($n = 5$)	HP (–) control ($n = 2$)	HP (–) Cancer ($n = 2$)	HP (+) control ($n = 3$)	HP (+) Cancer ($n = 5$)
Streptococcus pseudopneumoniae	0.139	**18.68**	3.375	6.038	3.797	**23.11**	0.983	0.196
Haemophilus parainfluenzae	0.155	3.842	0.457	0.959	2.154	2.413	0.130	0.018
S. oralis	0.023	**1.724**	0.145	0.297	0.500	**2.073**	0.078	0.009
S. parasanguinis	0.138	**1.506**	0.275	0.596	0.855	**3.361**	0.135	0.042
H. influenzae	0.000	0.433	0.016	0.034	0.000	0.408	0.000	0.000
Enterococcus hirae	0.000	0.261	0.000	0.000	0.000	0.011	0.000	0.000
Lactobacillus fermentum	0.000	0.209	0.000	0.000	0.000	0.084	0.000	0.030
HP	0.107	0.111	86.470	60.305	0.419	0.096	89.777	75.932
Klebsiella pneumoniae	0.000	0.068	0.000	1.054	0.000	0.017	0.000	0.030
Staphylococcus epidermidis	0.084	0.043	0.047	0.468	0.009	0.008	0.000	0.030
Enterobacter aerogenes	0.000	0.035	0.000	0.004	0.000	0.000	0.000	0.012
L. gasseri	0.000	0.026	0.000	0.025	0.000	0.008	0.000	0.011
Citrobacter rodentium	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000
S. pneumoniae	0.006	0.008	0.201	0.008	0.017	0.019	0.133	0.000
E. mori	0.000	0.000	0.000	0.000	0.009	0.017	0.000	0.000

Values are expressed as mean percent. Bold style means cutoff >1.0

modifications induced by urease producing bacteria (UB) may modify bacterial substrate availability and local immune responses by relationship between their members. Nitrosating bacteria (NB) concentrations were significantly higher when pH was >4 [66]. Nitrosating capacity was higher in a range from pH 3 to pH 6 [67]. UB-NB interaction was able to produce a pro-carcinogenic inflammatory response like as *H. pylori* [17]. Surprisingly, the proportion of UB and NB was significantly higher in the *H. pylori*-negative GC group (Table 59.2, Fig. 59.8), especially in the corpus mucosa [17] (Fig. 59.9). When we assessed the overlap of non-*H. pylori*-UB and non-*H. pylori*-NB, it revealed that the composition of *S. pseudopneumoniae*, *S. parasanguinis*, and *S. oralis* was higher in *H. pylori*-negative GC groups than the others. The *S. mitis* groups such as *S. mitis, S. pseudopneumoniae, S. oralis*, *S. infantis,* and *S. tigurinus* strains were associated with serious invasive infections, pneumonia, and endocarditis [68–70]. *S. mitis* is significantly more prevalent within esophageal carcinoma tissues [71]. Additionally, *S. parasanguinis* strain was associated with cystic fibrosis [72]. Taken together these species could be a significant human pathogen contributing to the development of *H. pylori*-negative GC.

We also found these similar findings in the patients with remote past *H. pylori* infection without history of *H. pylori* eradication [57]. In this group, the relative abundance of *Streptococcus, Parvimonas*, and Lactobacillales increased [57]. Due to hypochlorhydria by

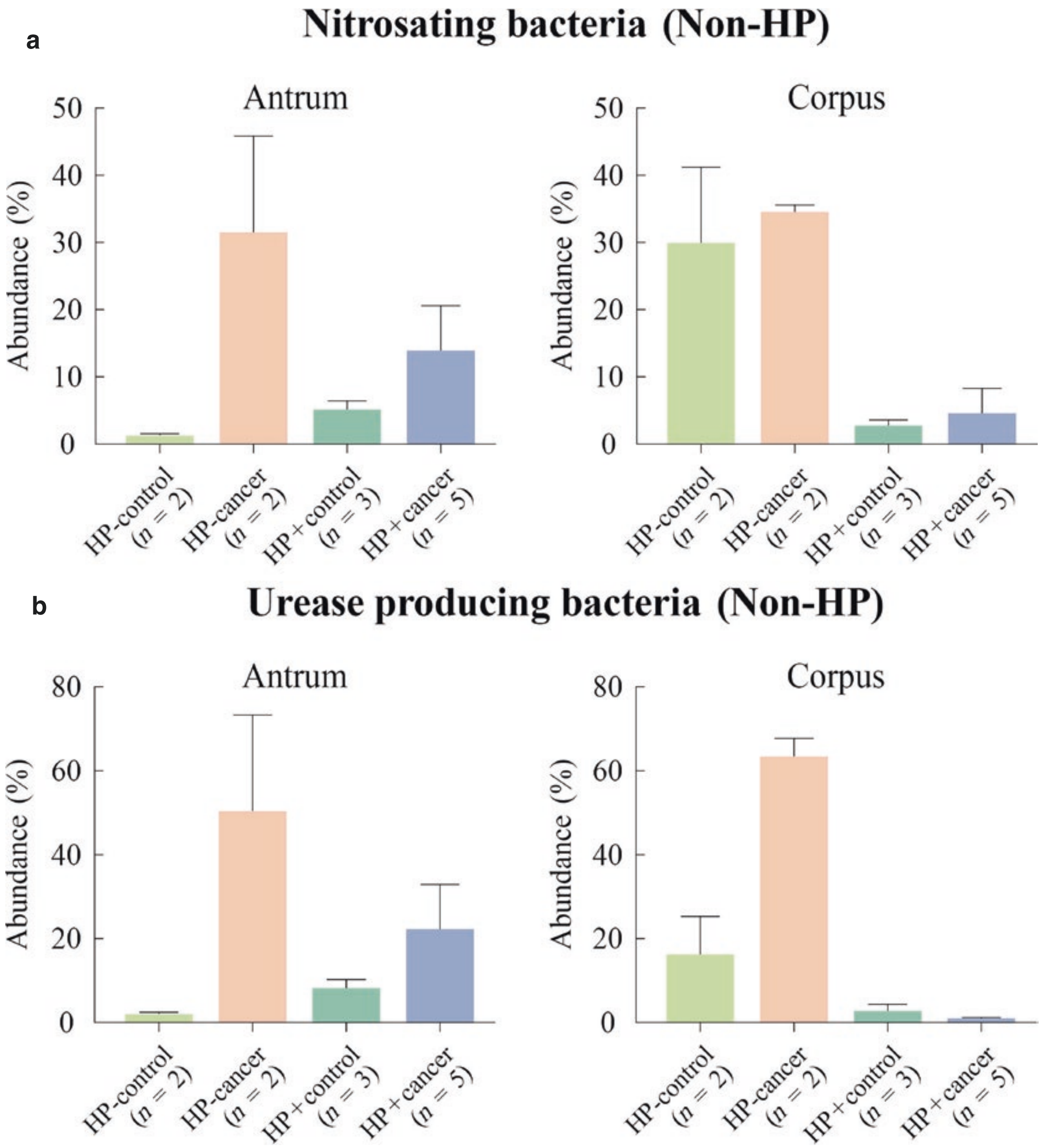
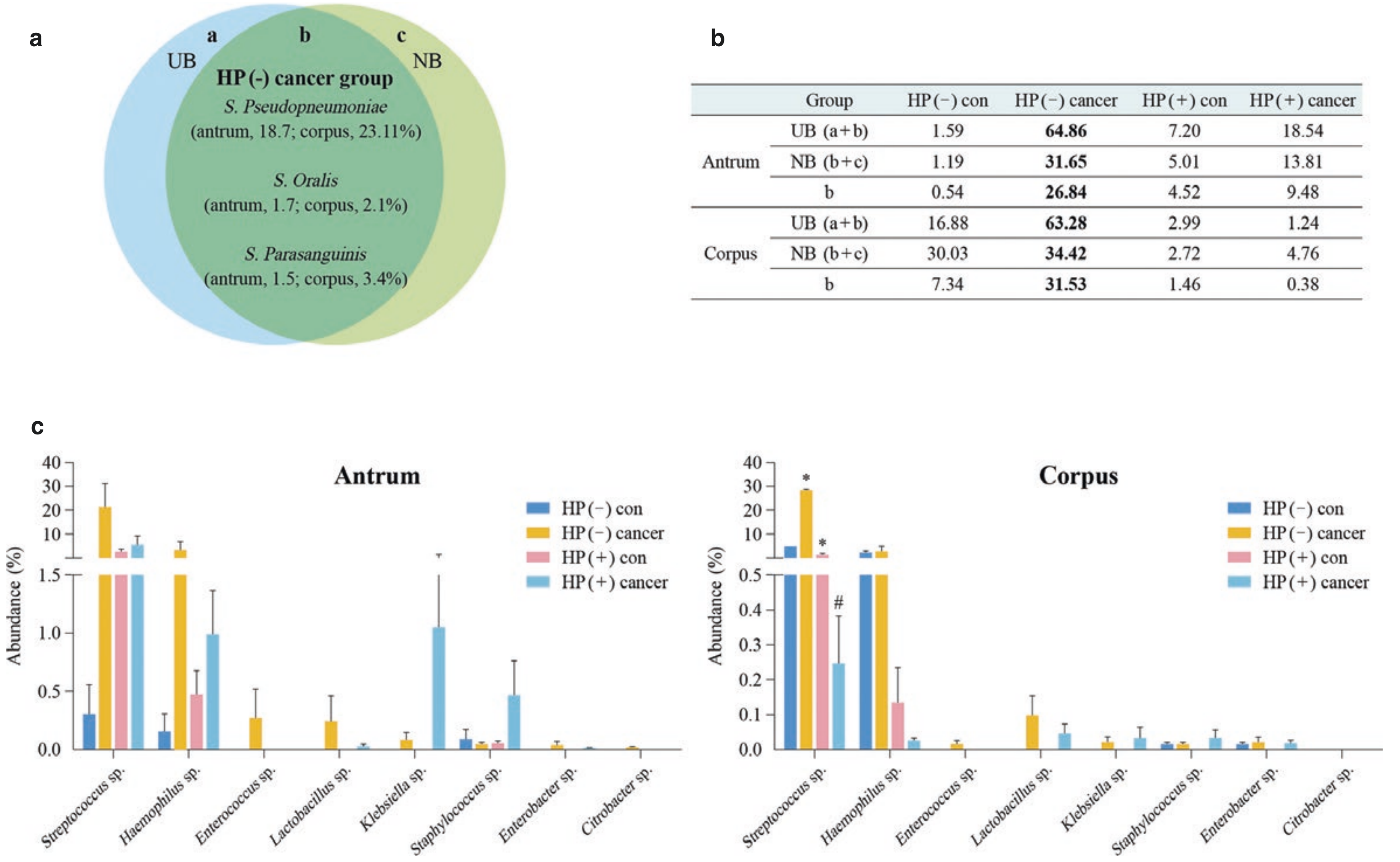


Fig. 59.8 The comparison of gastric microbiome in gastric antrum and corpus mucosa. (**a**) The proportion of non-*H. pylori* nitrosating bacteria (non-HP NB) between the gastric antrum and corpus mucosa. (**b**) The proportion of non-*H. pylori* urease producing bacteria (non-HP UB) between the gastric antrum and corpus mucosa. *HP H. pylori*; *NB* nitrosating bacteria; *UB* urease producing bacteria (Adapted from Sohn et al. [17])

mucosal atrophy, many oral cavity bacteria can colonise the stomach, and thus the gastric microbiota becomes similar to oral cavity microbiota [38]. Furthermore, the relative abundance of both non-*H. pylori*-UB as well as non-*H. pylori*-NB significantly increased in this group [57] which is rather similar to that of *H. pylori*-negative GC.



	Group	HP(−) con	HP(−) cancer	HP(+) con	HP(+) cancer
Antrum	UB (a+b)	1.59	**64.86**	7.20	18.54
	NB (b+c)	1.19	**31.65**	5.01	13.81
	b	0.54	**26.84**	4.52	9.48
Corpus	UB (a+b)	16.88	**63.28**	2.99	1.24
	NB (b+c)	30.03	**34.42**	2.72	4.76
	b	7.34	**31.53**	1.46	0.38

Fig. 59.9 The comparison of urease and N-nitroso compounds-producing bacteria in gastric antrum and corpus mucosa. (**a**) The strains of the overlap of non-HP-UB and non-HP-NB between the gastric antrum and corpus mucosa. (**b**) The proportion of UB and NB was higher in the HP-negative cancer than the other groups especially in the corpus. (**c**) Kruskal-Wallis test was used to determine statistical significance existed between the four groups. *$p < 0.05$ compared to HP(−) control; #$p < 0.05$ compared to HP(−) cancer. *HP*, *H. pylori*; *NB* nitrosating bacteria; *UB* urease producing bacteria (Adapted from Sohn et al. [17])

59.6.2 Bacterial Metabolites

Intestinal microbial dysbiosis has been associated with cellular immunity and immune function, which affects the GC development [73, 74]. NOD-like receptors (NLRs) and toll-like receptors (TLRs) [75] can bridge this interplay, eventually promoting carcinogenesis in a chronic process. TLRs critically affect the innate immune system, assuming their capability in differentiating host molecules from microbial molecules. NLRs adjust the innate immune response, correspondingly activating inflammasome mediated dysbiosis and modulating microbial composition. The gut microbiota can generate butyrate, which can differentiate regulatory T cells and IL-10, generating T cells via the activation of histone deacetylase inhibition (HDACi), interactions with G protein-coupled receptor 43 (GPR43), and IL-10 up-regulation [76]. Lipoteichoic acid (LTA) and short chain fatty acids (SCFAs) have opposite roles in carcinogenesis [77]. LTA accelerates malignant transformation. In contrast, SCFAs can mediate immunoregulation by Tregs, hence showing anti-carcinogenic and anti-inflammatory effects [76, 78, 79]. TLRs can develop GI tract tumors by activating the STAT3 and NF-κB signaling pathways [75]. The TLR4 activation, the receptor for LPS generated by the gut microbiome in the epithelial cells, can induce tumor development by up-regulating prostaglandin-endoperoxide synthase 2 (PTGS2) and activating the epidermal growth factor receptor (EGFR) signaling pathway in mice receiving azoxymethane [80]. In addition, the activation of the STAT3 signaling pathway up-regulates the expression of TLR2 in gastric epithelial cells, promoting tumor development in mice stomach [81].

59.6.3 Bacterial Genotoxins

It has been shown that intestinal bacteria are able to potentiate carcinogenesis by the specific toxins inducing DNA damage. As the disturbed microbes overgrow, they increase accumulating endotoxins and exotoxins, like cytolethal distending toxin from Shigella dysenteriae, cytolethal distending toxin and colibactin from *E. coli*, hydrogen peroxide, extracellular superoxide from Enterococcus faecalis, Enterotoxigenic *B. fragilis* toxin, a virulence factor activating the NF-κB and WNT signaling pathways in epithelial cells [82–84], from *B. fragilis*, etc. These toxins directly or indirectly cause genomic instability, DNA damage, and the invasion of adenocarcinomas [85–88]. Colibactin in *E. coli* can induce DNA damage, influence genomic instability [85, 89], and promote carcinogenesis. Colibactin-producing *K. pneumoniae* can induce chronic inflammation, DNA damage, and mutation [90]. In addition, cytolethal distending toxin produced by the *Helicobacter* species and *E. coli* can induce DNA damage in mammalian cells [91–94]. Finally, the accumulation of base excision repair intermediates and unrepaired DNA cause genomic instability and carcinogenesis [95, 96].

59.7 Conclusions

It was known that various bacteria that have not been cultured exist in the stomach as the molecular biological technologies develop. Although various studies have been conducted, the research designs are different from each other. Therefore, it is not easy to compare uniformly. However, many researchers tried to solve the link between the gastric microbiota and GC. There are many components which make the healthy stomach to become tumor microenvironment where GC easily develop. The microbiota itself or the microbial metabolites/bacterial genotoxins interplay and cooperate in the gastric carcinogenesis by way of immune mechanism [25].

The protein analysis study, proteomics, should be done to uncover about the actual function of the detected bacteria. A better understanding of the gastric microbial communities in health [97] and disease would shed light on the pathogenesis, diagnosis, treatment, and prevention of gastric illnesses.

References

1. Yang I, Nell S, Suerbaum S. Survival in hostile territory: the microbiota of the stomach. FEMS Microbiol Rev. 2013;37:736–61.
2. Brawner KM, Morrow CD, Smith PD. Gastric microbiome and gastric cancer. Cancer J. 2014;20:211–6.
3. Bik EM, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, et al. Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci U S A. 2006;103:732–7.
4. Andersson AF, Lindberg M, Jakobsson H, Bäckhed F, Nyrén P, Engstrand L. Comparative analysis of human gut microbiota by barcoded pyrosequencing. PLoS One. 2008;3:e2836.
5. Delgado S, Cabrera-Rubio R, Mira A, Suárez A, Mayo B. Microbiological survey of the human gastric ecosystem using culturing and pyrosequencing methods. Microb Ecol. 2013;65:763–72.
6. Eun CS, Kim BK, Han DS, Kim SY, Kim KM, Choi BY, et al. Differences in gastric mucosal microbiota profiling in patients with chronic gastritis, intestinal metaplasia, and gastric cancer using pyrosequencing methods. Helicobacter. 2014;19:407–16.
7. Winter SE, Winter MG, Xavier MN, Thiennimitr P, Poon V, Keestra AM, et al. Host-derived nitrate boosts growth of *E. Coli* in the inflamed gut. Science. 2013;339:708–11.
8. Martinsen TC, Bergh K, Waldum HL. Gastric juice: a barrier against infectious diseases. Basic Clin Pharmacol Toxicol. 2005;96:94–102.
9. Tennant SM, Hartland EL, Phumoonna T, Lyras D, Rood JI, Robins-Browne RM, et al. Influence of gastric acid on susceptibility to infection with ingested bacterial pathogens. Infect Immun. 2008;76:639–45.
10. Jo HJ, Kim J, Kim N, Park JH, Nam RH, Seok YJ, et al. Analysis of gastric microbiota by pyrosequencing: minor role of bacteria other than *Helicobacter pylori* in the gastric carcinogenesis. Helicobacter. 2016;21:364–74.
11. Sung J, Kim N, Kim J, Jo HJ, Park JH, Nam RH, et al. Comparison of gastric microbiota between gastric juice and mucosa by next generation sequencing method. J Cancer Prev. 2016;21:60–5.
12. Amieva MR, El-Omar EM. Host-bacterial interactions in *Helicobacter pylori* infection. Gastroenterology. 2008;134:306–23.
13. von Rosenvinge EC, Song Y, White JR, Maddox C, Blanchard T, Fricke WF. Immune status, antibiotic medication and pH are associated with changes in the stomach fluid microbiota. ISME J. 2013;7:1354–66.
14. Wu WM, Yang YS, Peng LH. Microbiota in the stomach: new insights. J Dig Dis. 2014;15:54–61.
15. Sachs G, Weeks DL, Melchers K, Scott DR. The gastric biology of *Helicobacter pylori*. Annu Rev Physiol. 2003;65:349–69.
16. Li XX, Wong GL, To KF, Wong VW, Lai LH, Chow DK, et al. Bacterial microbiota profiling in gastritis without *Helicobacter pylori* infection or nonsteroidal anti-inflammatory drug use. PLoS One. 2009;4:e7985.
17. Sohn SH, Kim N, Jo HJ, Kim J, Park JH, Nam RH, et al. Analysis of gastric body microbiota by pyrosequencing: possible role of bacteria other than *Helicobacter pylori* in the gastric carcinogenesis. J Cancer Prev. 2017;22:115–25.
18. Kawamura Y, Whiley RA, Shu SE, Ezaki T, Hardie JM. Genetic approaches to the identification of the mitis group within the genus streptococcus. Microbiology. 1999;145:2605–13.
19. Kawamura Y, Hou XG, Todome Y, Sultana F, Hirose K, Shu SE, et al. *Streptococcus peroris* sp. nov. and *streptococcus infantis* sp. nov., new members of the *Streptococcus mitis* group, isolated from human clinical specimens. Int J Syst Bacteriol. 1998;48:921–7.
20. Dhotre SV, Mehetre GT, Dharne MS, Suryawanshi NM, Nagoba BS. Isolation of *streptococcus tigurinus*: a novel member of *Streptococcus mitis* group from a case of periodontitis. FEMS Microbiol Lett. 2014;357:131–5.
21. Williams C, McColl KE. Review article: proton pump inhibitors and bacterial overgrowth. Aliment Pharmacol Ther. 2006;23:3–10.
22. Engstrand L, Lindberg M. *Helicobacter pylori* and the gastric microbiota. Best Pract Res Clin Gastroenterol. 2013;27:39–45.
23. Zilberstein B, Quintanilha AG, Santos MA, Pajecki D, Moura EG, Alves PR, et al. Digestive tract microbiota in healthy volunteers. Clinics (Sao Paulo). 2007;62:47–54.
24. Ginsburg I. Role of lipoteichoic acid in infection and inflammation. Lancet Infect Dis. 2002;2:171–9.
25. Bakhti SZ, Latifi-Navid S. Interplay and cooperation of *Helicobacter pylori* and gut microbiota in gastric carcinogenesis. BMC Microbiol. 2021;21:258.
26. Maldonado-Contreras A, Goldfarb KC, Godoy-Vitorino F, Karaoz U, Contreras M, Blaser MJ, et al. Structure of the human gastric bacterial community in relation to *Helicobacter pylori* status. ISME J. 2011;5:574–9.
27. Miftahussurur M, Waskito LA, El-Serag HB, Ajami NJ, Nusi IA, Syam AF, et al. Gastric microbiota and *Helicobacter pylori* in Indonesian population. Helicobacter. 2020;25:e12695.
28. Llorca L, Perez-Perez G, Urruzuno P, Martinez MJ, Iizumi T, Gao Z, et al. Characterization of the gastric microbiota in a pediatric population according to *Helicobacter pylori* status. Pediatr Infect Dis J. 2017;36:173–8.
29. Oh JD, Kling-Bäckhed H, Giannakis M, Engstrand LG, Gordon JI. Interactions between gastric epithelial stem cells and *Helicobacter pylori* in the setting of chronic atrophic gastritis. Curr Opin Microbiol. 2006;9:21–7.
30. Bury-Moné S, Mendz GL, Ball GE, Thibonnier M, Stingl K, Ecobichon C, et al. Roles of alpha and beta carbonic anhydrases of *Helicobacter pylori* in the urease-dependent response to acidity and in colonization of the murine gastric mucosa. Infect Immun. 2008;76:497–509.
31. Tan MP, Kaparakis M, Galic M, Pedersen J, Pearse M, Wijburg OL, et al. Chronic *Helicobacter pylori*

infection does not significantly alter the microbiota of the murine stomach. Appl Environ Microbiol. 2007;73:1010–3.

32. Martin ME, Bhatnagar S, George MD, Paster BJ, Canfield DR, Eisen JA, et al. The impact of *Helicobacter pylori* infection on the gastric microbiota of the rhesus macaque. PLoS One. 2013;8:e76375.
33. Kim J, Kim N, Jo HJ, Park JH, Nam RH, Seok YJ, et al. An appropriate cutoff value in the determination of colonization of *Helicobacter pylori* by pyrosequencing method: comparison to conventional methods. Helicobacter. 2015;20:370–80.
34. Dicksved J, Lindberg M, Rosenquist M, Enroth H, Jansson JK, Engstrand L. Molecular characterization of the stomach microbiota in patients with gastric cancer and in controls. J Med Microbiol. 2009;58:509–16.
35. Coker OO, Dai Z, Nie Y, Zhao G, Cao L, Nakatsu G, et al. Mucosal microbiome dysbiosis in gastric carcinogenesis. Gut. 2018;67:1024–32.
36. Ferreira RM, Pereira-Marques J, Pinto-Ribeiro I, Costa JL, Carneiro F, Machado JC, et al. Gastric microbial community profiling reveals a dysbiotic cancer-associated microbiota. Gut. 2018;67:226–36.
37. Bakhti SZ, Latifi-Navid S. Oral microbiota and *Helicobacter pylori* in gastric carcinogenesis: what do we know and where next? BMC Microbiol. 2021;21:71.
38. Sheh A, Fox JG. The role of the gastrointestinal microbiome in *Helicobacter pylori p*athogenesis. Gut Microbes. 2013;4:505–31.
39. Li J, Perez-Perez GI. *Helicobacter pylori* the latent human pathogen or an ancestral commensal organism. Front Microbiol. 2018;9:609.
40. Gao J-J, Zhang Y, Gerhard M, Mejias-Luque R, Zhang L, Vieth M, et al. Association between gut microbiota and *Helicobacter pylori*-related gastric lesions in a high-risk population of gastric cancer. Front Cell Infect Microbiol. 2018;8:202.
41. Dash NR, Khoder G, Nada AM, Al Bataineh MT. Exploring the impact of *Helicobacter pylori* on gut microbiome composition. PLoS One. 2019;14:e0218274.
42. Dias-Jácome E, Libânio D, Borges-Canha M, Galaghar A, Pimentel-Nunes P. Gastric microbiota and carcinogenesis: the role of non-*Helicobacter pylori* bacteria—A systematic review. Rev Esp Enferm Dig. 2016;108:530–40.
43. Wang LL, Yu XJ, Zhan SH, Jia SJ, Tian ZB, Dong QJ. Participation of microbiota in the development of gastric cancer. World J Gastroenterol. 2014;20:4948–52.
44. Yang L, Zhang J, Xu J, Wei X, Yang J, Liu Y, et al. *Helicobacter pylori* infection aggravates dysbiosis of gut microbiome in children with gastritis. Front Cell Infect Microbiol. 2019;9:375.
45. Vinasco K, Mitchell HM, Kaakoush NO, Castano-Rodriguez N. Microbial carcinogenesis: lactic acid bacteria in gastric cancer. Biochim Biophys Acta Rev Cancer. 2019;1872:188309.
46. Yang J, Zhou X, Liu X, Ling Z, Ji F. Role of the gastric microbiome in gastric cancer: from carcinogenesis to treatment. Front Microbiol. 2021;12:641322.
47. Beigier-Bompadre M, Moos V, Belogolova E, Allers K, Schneider T, Churin Y, et al. Modulation of the CD4 + T-cell response by *Helicobacter pylori* depends on known virulence factors and bacterial cholesterol and cholesterol α-glucoside content. J Infect Dis. 2011;204:1339–48.
48. Bimczok D, Clements R, Waites K, Novak L, Eckhoff D, Mannon P, et al. Human primary gastric dendritic cells induce a Th1 response to *H. pylori*. Mucosal Immunol. 2010;3:260–9.
49. Pereira-Marques J, Ferreira RM, Pinto-Ribeiro I, Figueiredo C. *Helicobacter pylori* infection, the gastric microbiome and gastric cancer. In: Kamiya S, Backert S, editors. *helicobacter pylori* in human diseases. advances in experimental medicine and biology, vol. 1149. Cham: Springer; 2019. p. 195–210.
50. Wang L, Xin Y, Zhou J, Tian Z, Liu C, Yu X, et al. Gastric mucosa-associated microbial signatures of early gastric cancer. Front Microbiol. 2020;11:1548.
51. Giannella RA, Broitman SA, Zamcheck N. Gastric acid barrier to ingested microorganisms in man: studies in vivo and in vitro. Gut. 1972;13:251–6.
52. Mowat C, Williams C, Gillen D, Hossack M, Gilmour D, Carswell A, et al. Omeprazole, *Helicobacter pylori* status, and alterations in the intragastric milieu facilitating bacterial *N*-nitrosation. Gastroenterology. 2000;119:339–47.
53. Kodama K, Sumii K, Kawano M, Kido T, Nojima K, Sumii M, et al. Gastric juice nitrite and vitamin C in patients with gastric cancer and atrophic gastritis: is low acidity solely responsible for cancer risk? Eur J Gastroenterol Hepatol. 2003;15:987–93.
54. Leach SA, Thompson M, Hill M. Bacterially catalysed *N*-nitrosation reactions and their relative importance in the human stomach. Carcinogenesis. 1987;8:1907–12.
55. Stockbruegger RW. Bacterial overgrowth as a consequence of reduced gastric acidity. Scand J Gastroenterol Suppl. 1985;111:7–16.
56. Lertpiriyapong K, Whary MT, Muthupalani S, Lofgren JL, Gamazon ER, Feng Y, et al. Gastric colonisation with a restricted commensal microbiota replicates the promotion of neoplastic lesions by diverse intestinal microbiota in the *Helicobacter pylori* INS-GAS mouse model of gastric carcinogenesis. Gut. 2014;63:54–63.
57. Shin CM, Kim N, Park JH, Lee DH. Changes in gastric corpus microbiota with age and after *Helicobacter pylori* eradication: A long-term follow-up study. Front Microbiol. 2021;11:621879.
58. Sanduleanu S, Jonkers D, De Bruïne A, Hameeteman W, Stockbrügger RW. Double gastric infection with *Helicobacter pylori* and non-*Helicobacter pylori* bacteria during acid-suppressive therapy: increase of pro-inflammatory cytokines and development of atrophic gastritis. Aliment Pharmacol Ther. 2001;15:1163–75.

59. Ayanaba A, Alexander M. Microbial formation of nitrosamines in vitro. Appl Microbiol. 1973;25:862–8.
60. Gobert AP, Mersey BD, Cheng Y, Blumberg DR, Newton JC, Wilson KT. Cutting edge: urease release by *Helicobacter pylori* stimulates macrophage inducible nitric oxide synthase. J Immunol. 2002;168:6002–6.
61. Suerbaum S, Michetti P. *Helicobacter pylori* infection. N Engl J Med. 2002;347:1175–86.
62. MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. Annu Rev Immunol. 1997;15:323–50.
63. Osaki T, Mabe K, Hanawa T, Kamiya S. Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of *Helicobacter pylori* infection. J Med Microbiol. 2008;57:814–9.
64. Brandi G, Biavati B, Calabrese C, Granata M, Nannetti A, Mattarelli P, et al. Urease-positive bacteria other than *Helicobacter pylori* in human gastric juice and mucosa. Am J Gastroenterol. 2006;101:1756–61.
65. Mobley HL, Hausinger RP. Microbial ureases: significance, regulation, and molecular characterization. Microbiol Rev. 1989;53:85–108.
66. Verdu E, Viani F, Armstrong D, Fraser R, Siegrist HH, Pignatelli B, et al. Effect of omeprazole on intragastric bacterial counts, nitrates, nitrites, and N-nitroso compounds. Gut. 1994;35:455–60.
67. Stockbrügger R, Armbrecht U. Bacterial overgrowth in the upper gastrointestinal-tract and possible consequences: report of a workshop in Brussels, Belgium, 9-10 February, 1990. Microb Ecol Health Dis. 1991;4:i–vii.
68. Shelburne SA, Sahasrabhojane P, Saldana M, Yao H, Su X, Horstmann N, et al. *Streptococcus mitis* strains causing severe clinical disease in cancer patients. Emerg Infect Dis. 2014;20:762–71.
69. Musher DM, Roig IL, Cazares G, Stager CE, Logan N, Safar H. Can an etiologic agent be identified in adults who are hospitalized for community-acquired pneumonia: results of a one-year study. J Infect. 2013;67:11–8.
70. Zbinden A, Mueller NJ, Tarr PE, Eich G, Schulthess B, Bahlmann AS, et al. *Streptococcus tigurinus*, a novel member of the *Streptococcus mitis* group, causes invasive infections. J Clin Microbiol. 2012;50:2969–73.
71. Narikiyo M, Tanabe C, Yamada Y, Igaki H, Tachimori Y, Kato H, et al. Frequent and preferential infection of *Treponema denticola, Streptococcus mitis* and *Streptococcus anginosus* in esophageal cancers. Cancer Sci. 2004;95:569–74.
72. Maeda Y, Elborn JS, Parkins MD, Reihill J, Goldsmith CE, Coulter WA, et al. Population structure and characterization of viridans group streptococci (VGS) including *Streptococcus pneumoniae* isolated from adult patients with cystic fibrosis (CF). J Cyst Fibros. 2011;10:133–9.
73. Vijay-Kumar M, Gewirtz A. Flagellin: key target of mucosal innate immunity. Mucosal Immunol. 2009;2:197–205.
74. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Eugene CY, Goodlett DR, et al. The innate immune response to bacterial flagellin is mediated by toll-like receptor 5. Nature. 2001;410:1099–103.
75. Rakoff-Nahoum S, Medzhitov R. Toll-like receptors and cancer. Nat Rev Cancer. 2009;9:57–63.
76. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013;341:569–73.
77. Brown DG, Rao S, Weir TL, O'Malia J, Bazan M, Brown RJ, et al. Metabolomics and metabolic pathway networks from human colorectal cancers, adjacent mucosa, and stool. Cancer Metab. 2016;4:11.
78. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013;504:446–50.
79. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, Deroos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013;504:451–5.
80. Fukata M, Chen A, Vamadevan AS, Cohen J, Breglio K, Krishnareddy S, et al. Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors. Gastroenterology. 2007;133(1869–9):e1814.
81. Tye H, Kennedy CL, Najdovska M, McLeod L, McCormack W, Hughes N, et al. STAT3-driven upregulation of TLR2 promotes gastric tumorigenesis independent of tumor inflammation. Cancer Cell. 2012;22:466–78.
82. Wu S, Morin PJ, Maouyo D, Sears CL. *Bacteroides fragilis* enterotoxin induces c-Myc expression and cellular proliferation. Gastroenterology. 2003;124:392–400.
83. Wu S, Rhee K-J, Zhang M, Franco A, Sears CL. *Bacteroides fragilis* toxin stimulates intestinal epithelial cell shedding and γ-secretase-dependent E-cadherin cleavage. J Cell Sci. 2007;120:1944–52.
84. Wu S, Powell J, Mathioudakis N, Kane S, Fernandez E, Sears CL. *Bacteroides fragilis* enterotoxin induces intestinal epithelial cell secretion of interleukin-8 through mitogen-activated protein kinases and a tyrosine kinase-regulated nuclear factor-κB pathway. Infect Immun. 2004;72:5832–9.
85. Cuevas-Ramos G, Petit CR, Marcq I, Boury M, Oswald E, Nougayrède J-P. *Escherichia coli* induces DNA damage in vivo and triggers genomic instability in mammalian cells. Proc Natl Acad Sci U S A. 2010;107:11537–42.
86. Arthur JC, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Fan T-J, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science. 2012;338:120–3.
87. Yamtich J, Nemec AA, Keh A, Sweasy JB. A germline polymorphism of DNA polymerase beta induces genomic instability and cellular transformation. PLoS Genet. 2012;8:e1003052.
88. Wang X, Huycke MM. Extracellular superoxide production by *enterococcus faecalis* promotes

chromosomal instability in mammalian cells. Gastroenterology. 2007;132:551–61.

89. Nougayrède J-P, Homburg S, Taieb F, Boury M, Brzuszkiewicz E, Gottschalk G, et al. *Escherichia coli* induces DNA double-strand breaks in eukaryotic cells. Science. 2006;313:848–51.
90. Kaur CP, Vadivelu J, Chandramathi S. Impact of *Klebsiella pneumoniae* in lower gastrointestinal tract diseases. J Dig Dis. 2018;19:262–71.
91. Nešić D, Hsu Y, Stebbins CE. Assembly and function of a bacterial genotoxin. Nature. 2004;429:429–33.
92. Ge Z, Rogers AB, Feng Y, Lee A, Xu S, Taylor NS, et al. Bacterial cytolethal distending toxin promotes the development of dysplasia in a model of microbially induced hepatocarcinogenesis. Cell Microbiol. 2007;9:2070–80.
93. Guidi R, Guerra L, Levi L, Stenerlöw B, Fox JG, Josenhans C, et al. Chronic exposure to the cytolethal distending toxins of gram-negative bacteria promotes genomic instability and altered DNA damage response. Cell Microbiol. 2013;15:98–113.
94. Graillot V, Dormoy I, Dupuy J, Shay JW, Huc L, Mirey G, et al. Genotoxicity of cytolethal distending toxin (CDT) on isogenic human colorectal cell lines: potential promoting effects for colorectal carcinogenesis. Front Cell Infect Microbiol. 2016;6:34.
95. Ray D, Kidane D. Gut microbiota imbalance and base excision repair dynamics in colon cancer. J Cancer. 2016;7:1421.
96. Brevik A, Joshi AD, Corral R, Onland-Moret NC, Siegmund KD, Le Marchand L, et al. Polymorphisms in base excision repair genes as colorectal cancer risk factors and modifiers of the effect of diets high in red meat. Cancer Epidemiol Biomark Prev. 2010;19:3167–73.
97. Vasapolli R, Schutte K, Schulz C, Vital M, Schomburg D, Pieper DH, et al. Analysis of transcriptionally active bacteria throughout the gastrointestinal tract of healthy individuals. Gastroenterology. 2019;157:1081–92.e3.

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The Effect of *H. pylori* Eradication on the Gastric Microbiota

Nayoung Kim

Abstract

Chronic infection of *Helicobacter pylori* (*H. pylori*) causes predominant occupation of *H. pylori* resulting in microbial dysbiosis of gastric microbiota. A growing number of studies have focused on gastric microbiota changes following *H. pylori* eradication but the results remain controversial whether successful *H. pylori* eradication could restore gastric microbiota to uninfected status. The microbial composition altered significantly after *H. pylori* eradication and the relative abundance of *H. pylori*-related taxa decreased. Accordingly, gastric commonly dominant commensals became enriched, and α-diversity indexes increased within 1 month after *H. pylori* eradication. Thus, significant differences in the gastric microbial community structure between before and after eradication were also seen within 1 month. Bioinformatic analyzes of microbiota functions showed that bacteria reproduction-related pathways were down-regulated and pathways of gastric acid secretion, etc. were up-regulated. Usually, the successful *H. pylori* eradication reverses the gastric microbiota dysbiosis and shows beneficial effects on gastric microbiota. However, the antibiotics which are used for *H. pylori* eradication inevitably change the gut microbiota outside of the stomach. A systemic review indicates that *H. pylori* eradication has minimal disruption of the gut microbiota so far.

Keywords

Helicobacter pylori · Eradication · Gastric
Gut · Microbiota · Pyrosequencing

60.1 Introduction

As the stomach is a hostile environment for most bacteria due to high acidity and various antimicrobial chemicals and enzymes, continuous colonization of any bacteria was thought to be difficult. Chronic infection of *Helicobacter pylori* (*H. pylori*) was regarded as awesome for a while. However, *H. pylori* infection has been confirmed to be associated with peptic ulcer diseases, low-grade gastric mucosa-associated lymphoma, and gastric cancer (GC) [1]. *H. pylori* eradication is recommended to prevent these diseases.

After next-generation sequencing (NGS) identified the domination by five phyla, including Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, and Proteobacteria [2, 3], there was keen interest regarding *H. pylori* colonization-

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associated alteration of gastric microbiota, and whether reduced microbial diversity could be restored by *H. pylori* eradication [4]. In the same time, there are concerns regarding dysbiosis caused by antibiotic therapy [5]. Even the results are controversial, systemic review and meta-analysis showed that *H. pylori* eradication has minimal disruption of gut microbiota [6]. In addition, successful *H. pylori* eradication potentially restores gastric microbiota to a similar status as found in uninfected individuals, and shows beneficial effects on gut microbiota [7]. In terms of antibiotic resistance of gut microbiota, Taiwanese study suggested little effect on the antibiotic resistance of *E. coli* [8]. In addition, there were significant improvements in metabolic parameters, with a decrease in insulin resistance, triglycerides, and low-density lipoprotein (LDL) and an increase in high-density lipoprotein (HDL) [8]. This decrease in insulin resistance could be related with the increase in glucagon-like peptide-1 (GLP-1) secretion which was suggested to be originated from alterations in gut microbiota, specifically *Lachnobacterium*, *Bifidobacterium adolescentis,* and Coriobacteriaceae [9]. These suggest that *H. pylori* eradication affect the gut microbiota as well as gastric microbiota. In addition, NGS research suggested that probiotic supplementation during and after eradication treatment could reduce the antibiotic-induced alteration and imbalance of the gut microbiota composition [10]. Furthermore, probiotics showed a possibility of restriction of the growth of antibiotic-resistant gut bacteria and increase of the *H. pylori* eradication success rate [10]. Consequently, there is no major argument of the long-term safety of *H. pylori* eradication therapy. In this chapter, the influence of successful *H. pylori* eradication on the short-term and long-term alterations of not only gastric microbiota but also gut microbiota was briefly reviewed.

60.2 Gastric Microbiota Changes in the Absence or in the Presence of *H. pylori* Infection

The main bacteria which affects the gastric microbiota is definitely *H. pylori*. Thus, most literature focused on the before and after *H. pylori* eradication. However, *H. pylori*–negative gastric microbiota might change as getting aged. Previously, we have shown that the lamina propria of rat gastric mucosa was replaced by connective tissue and characterized by the accumulation of oxidative products with aging [11]. In addition, rat aging has been associated with significantly decreased basal and stimulated gastric acid levels as well as with decreased expression of mRNA and protein of H^+-K^+-ATPase [12]. Thus, our team checked the gastric microbiota with age in the absence or in the presence of *H. pylori* with relation to gastric atrophy and or intestinal metaplasia (IM) over long follow-up (mean follow-up duration: 57.4 months [range: 12–145 months]) (Table 60.1) and there were much difference among four groups at the phylum level [5] (Fig. 60.1a). Four groups are as follows: the Group 1: *H. pylori*-uninfected subjects without evidence of atrophic gastritis (AG) and IM by histology, pepsinogen I/II ratio ≥ 4.0, and no history of *H. pylori* eradication ($n = 8$), Group 2: *H. pylori*-infected patients without mucosal atrophy and metaplasia by histology with pepsinogen I/II ratio > 4.0 ($n = 15$), Group 3: *H. pylori*-infected patients with AG and/or IM by histology and pepsinogen I/II ratio < 2.5 ($n = 17$), Group 4: the patients with remote past *H. pylori* infection without history of *H. pylori* eradication ($n = 3$) [5]. Compared with Group 1 (*H. pylori*-uninfected individuals), *H. pylori* predominates in the gastric corpus microbiota in Groups 2 & 3 (*H. pylori*-infected subjects),

Table 60.1 Baseline characteristics of the study subjects ($n = 43$)

	Group 1 HP (−), AG (−)	Group 2 HP (+), AG (−)	Group 3 HP (+), AG(+)	Group 4 HP (−), AG(+)	
	n = 8	*n* = 15	*n* = 17	*n* = 3	*p*-value
Male, n (%)	3 (37.5)	5 (33.3)	3 (17.6)	0 (0.0)	0.446
Age, y	49.6 ± 9.8	50.9 ± 11.7	58.3 ± 10.4	62.0 ± 10.4	0.092
FU duration, m, median (range)	93 (12–129)	44 (13–102)	81 (14–132)	56 (40–99)	NS
FU times, median (range)	2 (1–3)	1 (1–3)	2(1–4)	2 (1–3)	NS
Current smoker (*n* = 41), n (%)	2 (25.0)	1 (6.7)	0 (0.0)	0 (0.0)	0.163
Drinker, *n* (%)	3 (37.5)	4 (26.7)	1 (5.9)	0 (0.0)	0.194
Education					
Elementary-middle-high	7 (87.5)	4 (33.3)	11 (68.8)	3 (100)	0.034
University	1 (12.5)	8 (66.7)	5 (31.3)	0 (0.0)	
Histology (updated Sydney classification)					
HP					
Antrum	0.0 ± 0.0	1.6 ± 1.0	1.4 ± 0.9	0.0 ± 0.0	**< 0.001**
Corpus	0.0 ± 0.0	1.9 ± 0.9	2.1 ± 0.7	0.0 ± 0.0	**< 0.001**
Neutrophil infiltration					
Antrum	0.1 ± 0.4	1.9 ± 0.6	1.6 ± 0.9	0.0 ± 0.0	**< 0.001**
Corpus	0.1 ± 0.4	1.4 ± 0.9	2.1 ± 0.3	0.3 ± 0.6	**< 0.001**
Monocyte infiltration					
Antrum	0.0 ± 0.0	0.1 ± 0.3	1.3 ± 0.5	0.0 ± 0.0	**< 0.001**
Corpus	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.7	2.3 ± 0.6	**< 0.001**
AG					
Antrum	0.0 ± 0.0	0.1 ± 0.3	1.3 ± 0.5	0.0 ± 0.0	**< 0.001**
Corpus	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.7	2.3 ± 0.6	**< 0.001**
IM					
Antrum	0.0 ± 0.0	0.1 ± 0.3	1.2 ± 0.8	1.0 ± 0.0	**< 0.001**
Corpus	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.8	1.7 ± 0.6	**< 0.001**
PG I	50.6 ± 15.2	98.5 ± 91.6	45.7 ± 14.3	10.6 ± 10.3	**0.026**
PG II	9.0 ± 2.0	19.4 ± 10.8	22.2 ± 8.1	13.4 ± 6.6	**0.006**
PG I/II ratio	5.6 ± 0.8	5.5 ± 3.7	2.2 ± 0.8	0.7 ± 0.4	**< 0.001**

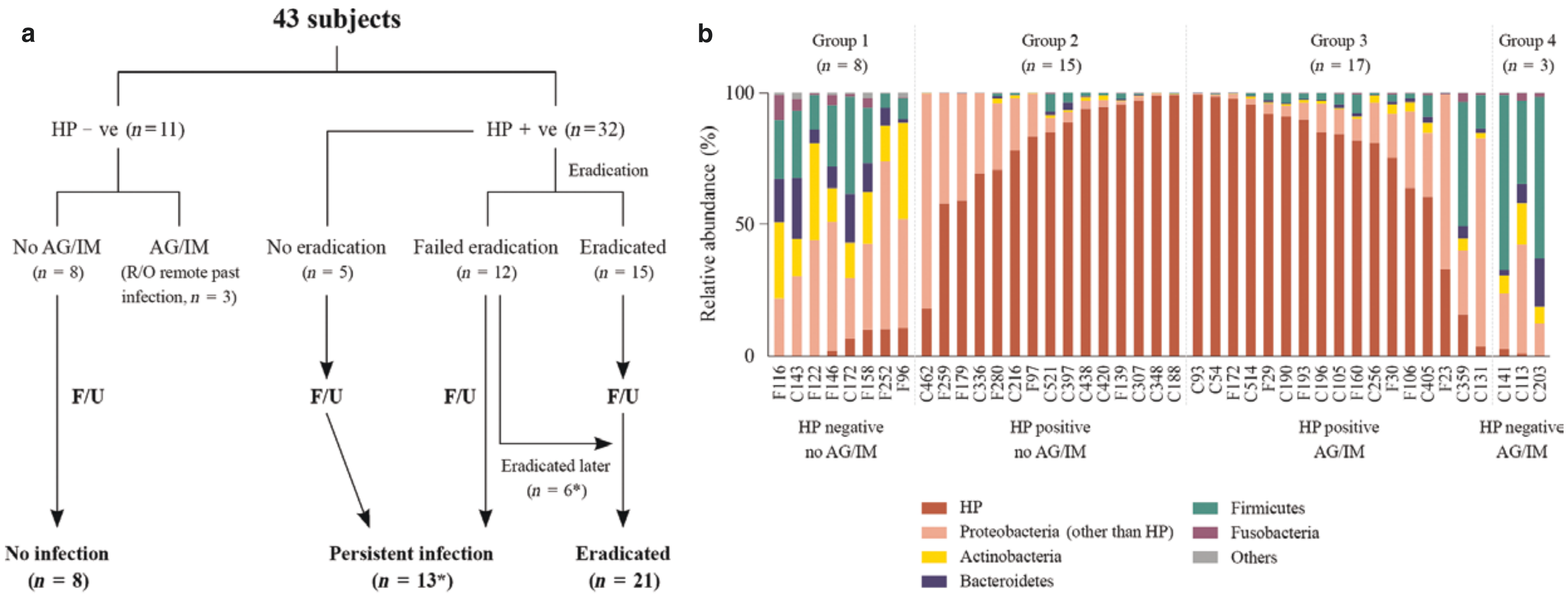
Adapted from Shin et al. [5]

p-values were calculated using the Chi-square test or Student's t-test. *HP Helicobacter pylori*, *AG* atrophic gastritis, *IM* intestinal metaplasia, *PG* pepsinogen, *FU* follow-up, *NS* non-significant

Group 4 (subjects with remote past *H. pylori* infection) (Fig. 60.2a).

60.2.1 Gastric Microbiota Changes in the Absence of *H. pylori* Infection

In the *H. pylori*-negative non-atrophy group (Group 1, *n* = 8), gastric corpus microbiota consisted of Proteobacteria (38.5%), Actinobacteria (23.3%), Firmicutes (21.2%), and Bacteroidetes (12.3%) (Fig. 60.2a). Their relative abundance of eight subjects during follow-up is shown at the phylum level (Fig. 60.3a). No individuals in Group 1 developed AG or IM during the follow-up period of up to 10 years. However, an increase in Proteobacteria abundance was observed during the follow-up (*p*-value for trend = 0.00002, Fig. 60.3b). Firmicutes and Fusobacteria showed a significant decrease with aging (*p* for trend = 0.001 and < 0.001, respectively



*Two patients had gastric mucosal samples after failed eradication as well as after sucessful eradication.

Fig. 60.1 Study flow chart and bacterial composition depending on *H. pylori* status. (**a**) There are four groups depending on *H. pylori* status. Group 1: *H. pylori*-uninfected subjects without evidence of atrophic gastritis (AG) and intestinal metaplasia (IM) by histology, pepsinogen I/II ratio ≥ 4.0, and no history of *H. pylori* eradication ($n = 8$), Group 2: *H. pylori*-infected patients without mucosal atrophy and metaplasia by histology with pepsinogen I/II ratio > 4.0 ($n = 15$), Group 3: *H. pylori*-infected patients with AG and/or IM by histology and pepsinogen I/II ratio < 2.5 ($n = 17$), Group 4: the patients with remote past *H. pylori* infection without history of *H. pylori* eradication ($n = 3$). (**b**) Bacterial composition of the study subjects ($n = 43$) at phylum level (Adapted from Shin et al. [5])

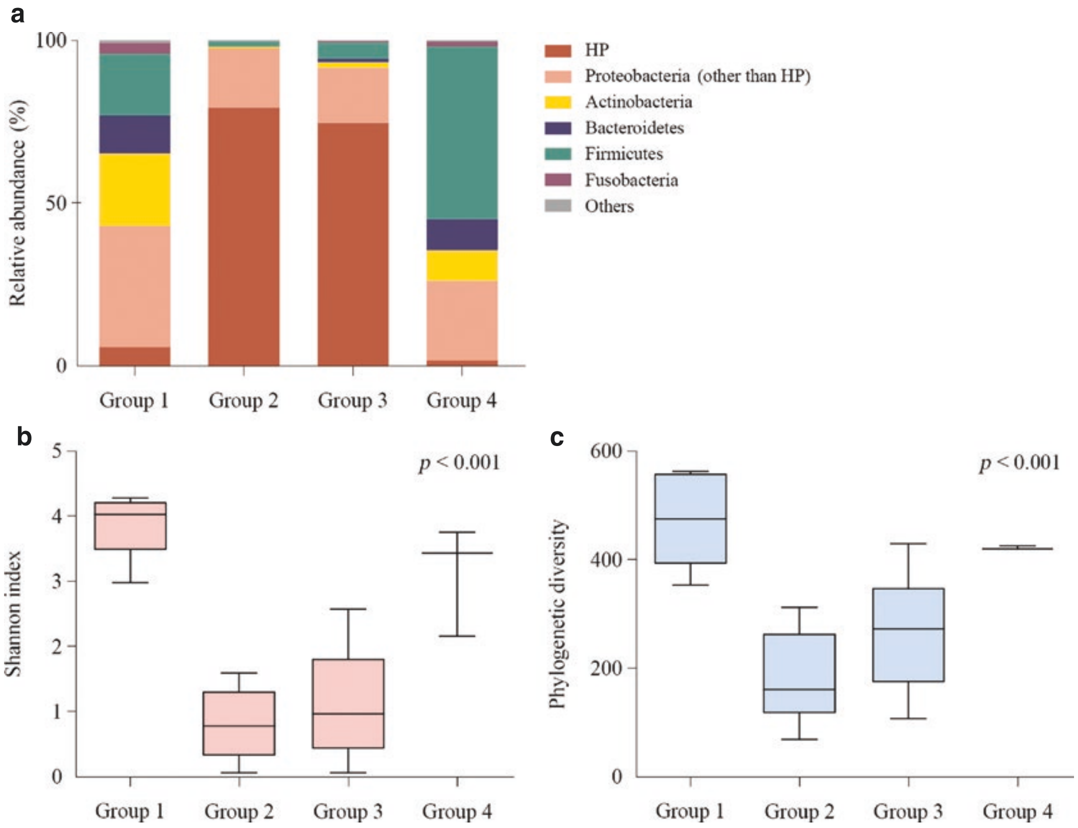


Fig. 60.2 Alteration of the gastric microbiota after *H. pylori* eradication. (**a**) As soon as *H. pylori* colonizes the stomach *H. pylori*-Proteobacteria becomes the dominant bacteria causing dysbiosis. After *H. pylori* eradication non-*H. pylori* Proteobacteria rapidly increases from 4.55% into 51.7% and the other Bacteroidetes, Fusobacteria, and Actinobacteria increases and dysbiosis disappears. (**b**) Compared with Group 1 (*H. pylori*-uninfected individuals), *H. pylori* predominates in the gastric corpus microbiota in Groups 2 & 3 (*H. pylori*-infected subjects), Group 4 (subjects with remote past *H. pylori* infection) the Shannon index is different. (c) Phylogenic index is similar to the Shannon index (Adapted from Shin et al. [5])

(Fig. 60.3c and Fig. 60.3d)). In addition, decreased number of microbial density was noted during the follow-up periods (p for trend = 0.0231 and 0.0018 by the observed operational taxonomic unit counts (OUT) and Shannon index, respectively (Fig. 60.3e and Fig. 60.3f)). The bacterial composition at the phylum level in eight individuals in Group 1 were very different from other three groups (Fig. 60.1b). When gastric microbiota profiles are compared between GC cases and controls, the effect of aging should be considered before the interpretation of the microbiome data. Our study suggests that age is not only a risk factor for GC but also has an effect on gastric microbiota [13].

(**a**) Relative abundance of 8 subjects during follow-up in the phylum level. (**b**) An increase in Proteobacteria abundance. (**c**) A decrease in Firmicutes. (**d**) A decrease in Fusobacterium. (**e**) A decrease of α-diversity indices (number of observed OTUs. (**f**) A decrease in Shannon index is also observed during the follow-up. p-values were calculated using general linear model. *OTUs,* operational taxonomy units.

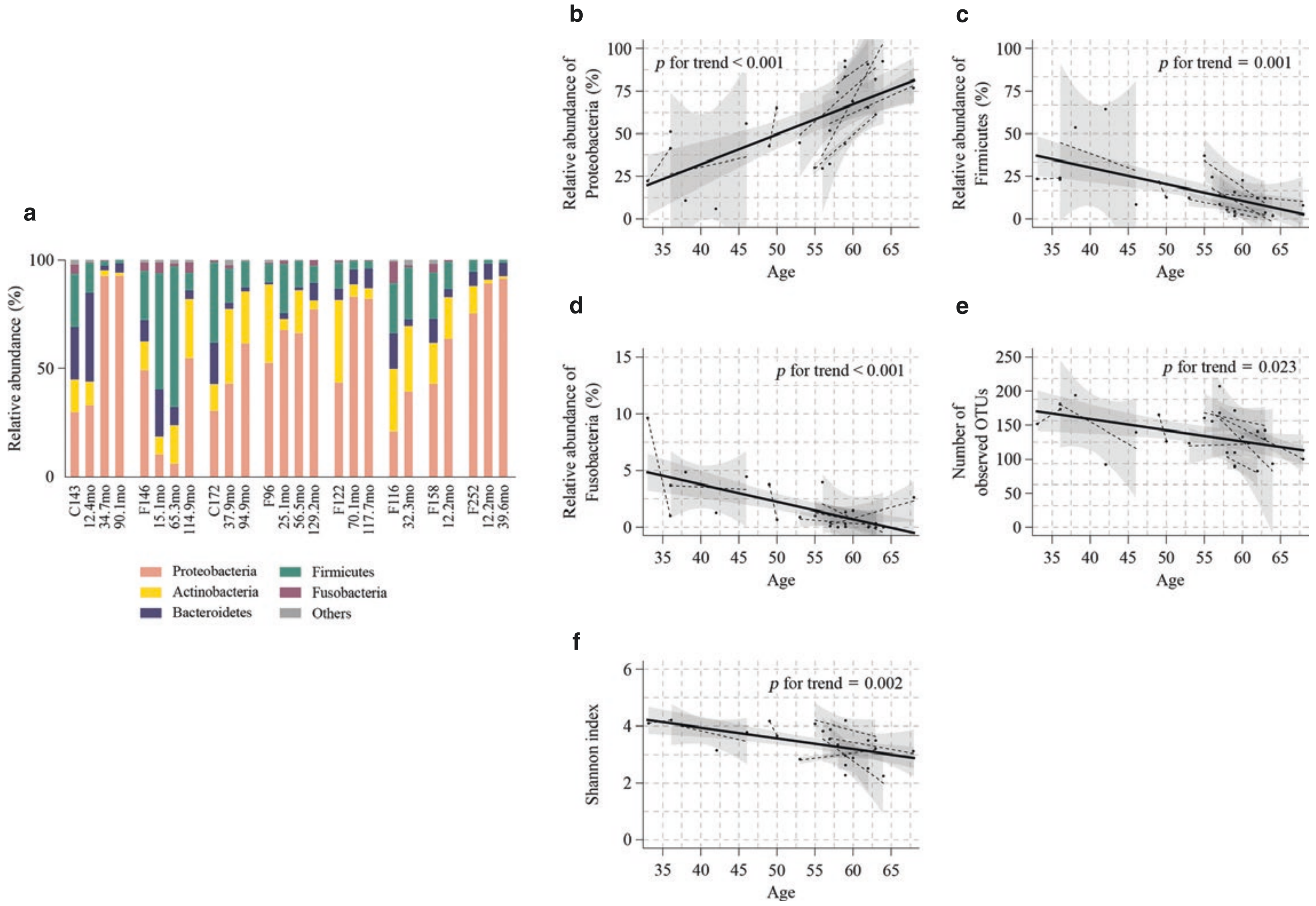


Fig. 60.3 Changes in gastric microbiota at the corpus in *H. pylori*-uninfected individuals without atrophic gastritis/intestinal metaplasia ($n = 8$) and their follow up changes ($n = 8$) (Adapted from Shin et al. [5])

60.2.2 Gastric Microbiota Changes in the Presence of *H. pylori* Infection

H. pylori infection changed the phylum composition (Fig. 60.2a) and significantly decreased the microbial diversity indices (Fig. 60.2b: Shannon index, Fig. 60.2c: phylogenetic diversity). Before *H. pylori* colonization, non-*H. pylori* Proteobacteria dominates the gastric microbiota without dysbiosis (Fig. 60.1a). The principal coordinates analysis (PCoA) plot of all study participants ($n = 43$, Bray-Curtis, Unweighted) is presented in Fig. 60.4a, which shows that the four groups were significantly different (PERMANOVA $p < 0.001$). In detail, *H. pylori*-positive groups (Groups 2 and 3) and *H. pylori*-negative groups (Groups 1 and 4) did not overlap with each other, but the four groups were not distinguished from each other by the presence or absence of AG or IM [5] (Fig. 60.4a). In addition, Linear discriminant analysis (LEfSe) analysis was performed to identify taxa that were significantly enriched in each group. Among subjects without atrophy or metaplasia (Groups 1 and 2, $n = 23$), *H. pylori* infection significantly changed mucosa-associated gastric microbiota in the corpus (decrease in Actinobacteria, Sphingomonadales, *Prevotella*, and *Veillonella* abundance; FDR $q < 0.05$ by LEfSe analysis of Group 1 vs. Group 2), which resulted in a decrease in microbial diversity ($p < 0.01$, Shannon and phylogenetic diversity indices [5] (Fig. 60.4b).

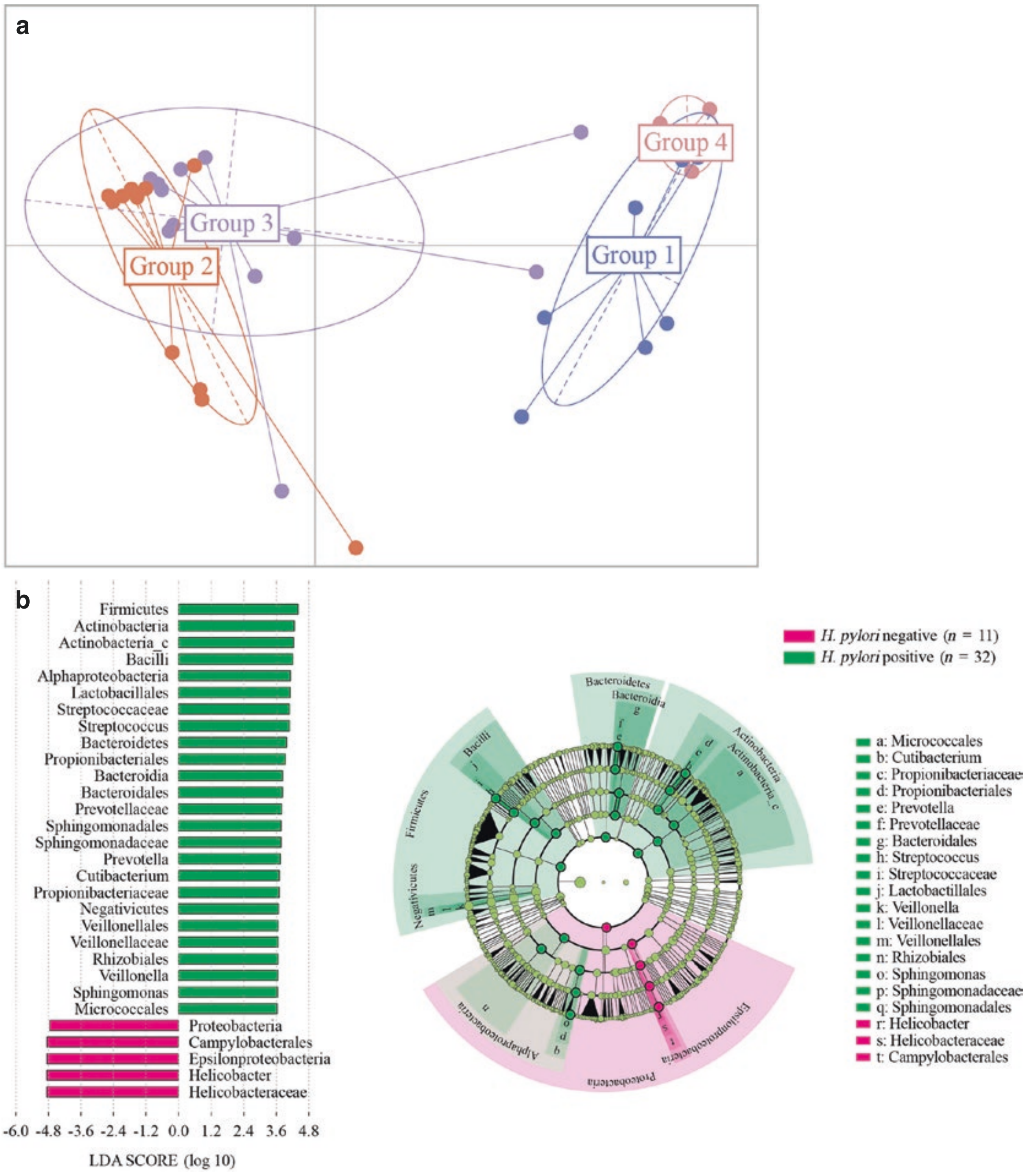


Fig. 60.4 The gastric microbial alteration after *H. pylori* infection (**a**) The principal coordinates analysis (PCoA) plot of all study participants ($n = 43$, Bray-Curtis, Unweighted). It shows that the 4 groups were significantly different (PERMANOVA $p < 0.001$). In detail, *H. pylori*-positive groups (Groups 2 and 3) and *H. pylori*-negative groups (Groups 1 and 4) did not overlap with each other, but the 4 groups were not distinguished from each other by the presence or absence of atrophic gastritis or intestinal metaplasia. (**b**) Linear discriminant analysis (LEfSe) analysis was performed to identify taxa that were significantly enriched in each group. Among subjects without atrophy or metaplasia (Groups 1 and 2, $n = 23$), *H. pylori* infection significantly changed mucosa-associated gastric microbiota in the corpus (decrease in Actinobacteria, Sphingomonadales, *Prevotella*, and *Veillonella* abundance; FDR $q < 0.05$ by LEfSe analysis of Group 1 vs. Group 2), which resulted in a decrease in microbial diversity ($p < 0.01$, Shannon and phylogenetic diversity indices) (Adapted from Shin et al. [5])

60.3 The Effect of *H. pylori* Eradication on the Gastric Microbiota

Usually, the effect of *H. pylori* eradication is basically analyzed in the aspect of gastric microbiota diversity such as α- and β-diversity, microbial composition such as phylum levels, gastric microbial interactions or functions depending on the short-term and long-term alterations. Although there is no clear definition of short- or long-term follow-up, usually short-term means one or several months, usually within 6 months. Another point is the presence or absence of atrophy/IM because gastric microbiota composition and diversity change by the atrophy/IM.

60.3.1 The Effect of *H. pylori* Eradication on the Gastric Microbiota Composition and Diversity

As soon as *H. pylori* colonizes the stomach, *H. pylori*-Proteobacteria becomes the dominant bacteria causing dysbiosis [5] (Fig. 60.5). After *H. pylori* eradication non-*H. pylori* Proteobacteria rapidly increases from 4.55% into 51.7% and the other Bacteroidetes, Fusobacteria, and Actinobacteria increases and dysbiosis disappears [5]. Naturally, α-diversity indexes reflecting the diversity within samples increased within 1 month after *H. pylori* eradication, including Chao 1 [14], Shannon [4, 15], and phylogenetic

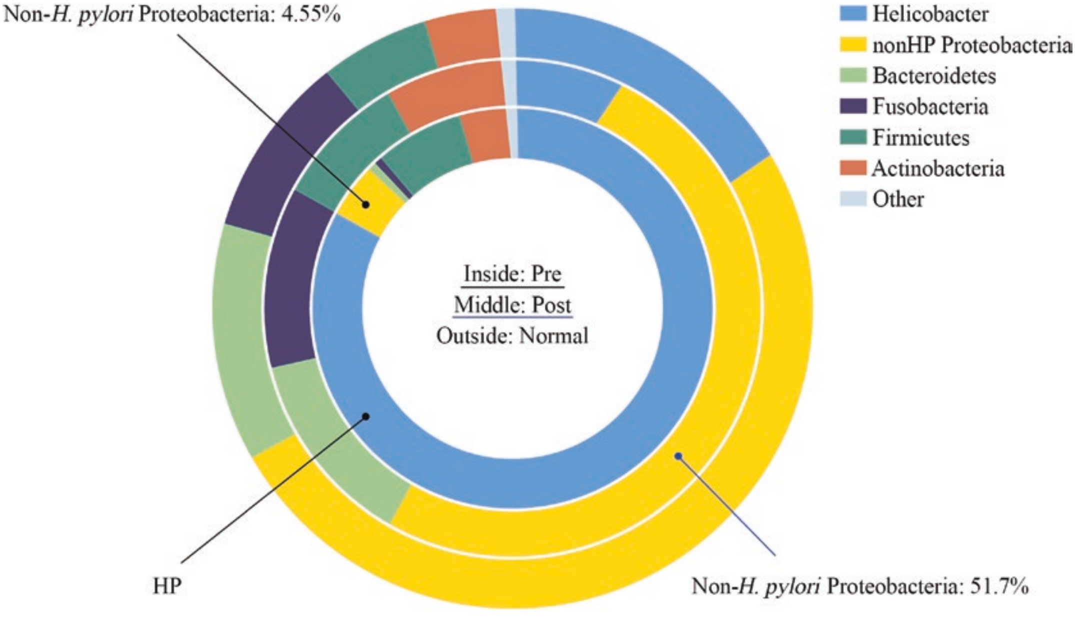


Fig. 60.5 The gastric microbial alteration after *H. pylori* infection and changes after *H. pylori* eradication. As soon as *H. pylori* colonizes the stomach *H. pylori*-Proteobacteria becomes the dominant bacteria causing dysbiosis. After *H. pylori* eradication non-*H. pylori* Proteobacteria rapidly increases from 4.55% into 51.7% and the other Bacteroidetes, Fusobacteria, and Actinobacteria increases and dysbiosis disappears (Adapted from Shin et al. [5])

diversity [4]. The trends of α-diversity changes persisted after follow-up of 6 months. That is, higher α-diversity indexes were seen compared to baseline, including richness index [7], Chao 1 [16], Shannon [17, 18], phylogenetic diversity [5], and Sobs index [16].

In terms of β-diversity reflecting diversity among samples, there were significant differences in gastric microbial community structure 1 month after *H. pylori* eradication [5]. In the long-term (≥ 6 months) follow-up, significant differences of β-diversity were also observed compared with that at baseline [7, 16]. Similar alterations of differences in beta diversity were also reported [5, 17, 18].

60.3.2 The Reversibility of Gastric Microbiota Composition and Diversity to that of *H. pylori*-Negative Individuals After *H. pylori* Eradication

Hong Kong group reported that *H. pylori* eradication resulted in an increase in bacterial diversity and restoration of the relative abundance of other bacteria to levels similar to individuals without *H. pylori* by Shannon (Fig. 60.6a) and phylogenetic diversity [4] (Fig. 60.6b). This recovery became similar to *H. pylori*-negative regardless of disease status such as gastritis, IM and GC by Shannon (Fig. 60.6c) and phylogenetic diversity [4] (Fig. 60.6d). Other studies showed the higher α−diversity compared with uninfected status 2 months after eradication [14, 19], and 6 months following eradication [7]. β−diversity also improved to the similar level of uninfected status [4, 15]. For longer follow-up, lower α−diversity and significant different β−diversity were reported [18]. However, when we investigated long-term changes in the gastric microbiota following *H. pylori* eradication ($n = 21$) this recovery of α-diversity after *H. pylori* eradication was determined by the pre-existing atrophy [5] (Table 60.2). That is, in 10 out of 21 patients (47.6%), the gastric microbiota profile appeared to be restored to that of *H. pylori*-negative individuals (non-dysbiosis group, Figs. 60.7a-c). However, in 11 patients (52.4%), severe dysbiosis with an increase in *Actinobacteria* abundance occurred, thus microbial diversity was not restored (dysbiosis group, Figs. 60.7d–f). Our findings suggest that *H. pylori* eradication does not always normalize the gastric microbiota [5]. The difference between the dysbiosis and non-dysbiosis groups could explain (Table 60.2). The proportion of individuals with AG/IM at baseline was significantly different between the two groups ($p = 0.011$ by χ^2-test). In addition, the increased abundance of *Acinetobacter* at baseline was significantly associated with dysbiosis after anti-*Helicobacter* treatment ($p = 0.005$, Table 60.2) [5]. One possible explanation is that persistent hypochlorhydria in subjects with severe AG even after *H. pylori* eradication may lead to colonization of the stomach with various bacteria, which can suppress the colonization of *Acinetobacter* species. In contrast, *H. pylori* eradication can restore normal gastric acid secretion in individuals without AG. Low gastric pH promotes an environment unfavorable for the survival of most bacteria. In contrast, some *Acinetobacter* species resistant to anti-*Helicobacter* treatment can survive and become predominant in this acidic environment. *Acinetobacter* species, such as *A. lwoffii* and *A. anitratu,* can produce urease under appropriate conditions [20]. A recent study has reported that the abundance of *Acinetobacter*, especially *A. lwoffii, A. rhizosphaerae*, and *A. guillouiae,* increased after *H. pylori* eradication [17]. An increase in *Acinetobacter* species abundance was associated with persistent gastric inflammation, and negatively correlated with the histologic grade of atrophy and IM after successful eradication, which is consistent with our findings [17]. These findings suggest that gastric dysbiosis after *H. pylori* eradication is not temporary but could persist for years. In addition, the colonization of *Acinetobacter* species in a subset of patients carries the potential risk for antibiotic-resistant bacterial infection [21, 22].

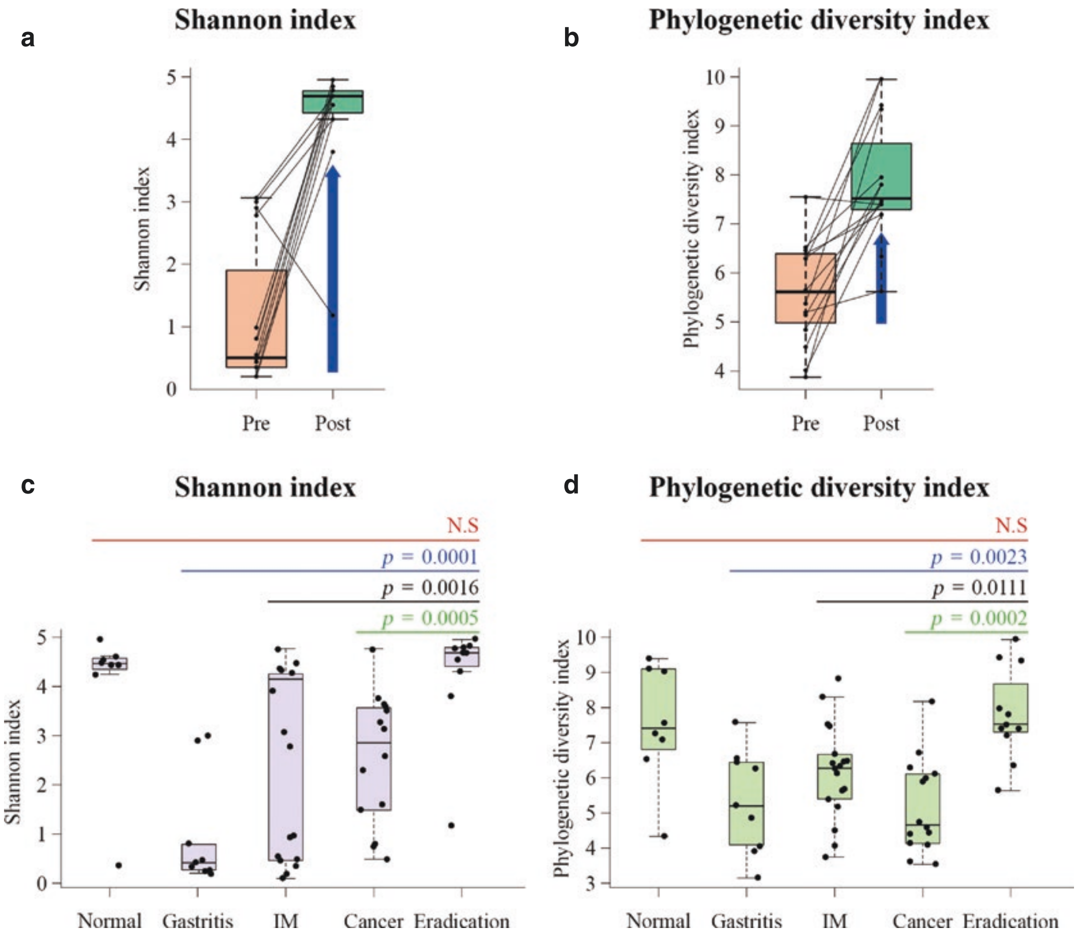


Fig. 60.6 α-diversity of gastric microbiota 8 weeks after *H. pylori* eradication. (**a**) Shannon index and (**b**) phylogenetic diversity index before and after *H. pylori* eradication. The black point indicates the sample, and the lines connected the paired samples of pre- and post-eradication. (**c**) Boxplot of Shannon index and (**d**) phylogenetic diversity index in different groups. The boxes denote interquartile ranges (IQR) with the median as a black line and whiskers extending up to the most extreme points within 1.5 fold IQR. The *p*-values between different groups were shown. *H. pylori* eradication elevated the diversity similar to the *H. pylori*-negative group and all different from gastritis, IM and cancer. *NS* non-significant, *IM* intestinal metaplasia (Adapted from Li et al. [4])

Table 60.2 Comparison of clinical variables, microbiota composition, and histology between the no dysbiosis group ($n = 10$) and the dysbiosis group ($n = 11$) after successful *H. pylori* eradication

	No dysbiosis	Dysbiosis	
	$n = 10$	$n = 11$	*p*-value
Male, *n* (%)	4 (40.0)	4 (36.4)	0.864
Age, years	58.3 ± 10.4	62.0 ± 10.4	0.092
Current smoker ($n = 20$), *n* (%)	0 (0.0)	1 (10.0)	1.000
Drinker, *n* (%)	2 (20.0)	2 (18.2)	1.000
AG or IM[a]	9 (90.0)	4 (36.4)	**0.011**
Relative abundance of *Acinetobacter* genus at baseline	1.3 ± 2.1	29.8 ± 26.5	**0.005**

(continued)

Table 60.2 (continued)

	No dysbiosis	Dysbiosis	
Histology at baseline (updated Sydney classification)			
H. pylori			
Antrum	1.5 ± 1.1	1.5 ± 0.7	0.909
Corpus	1.8 ± 0.8	1.8 ± 1.0	0.963
Neutrophil infiltration			
Antrum	1.5 ± 1.1	2.1 ± 0.3	0.125
Corpus	2.2 ± 0.4	1.6 ± 0.8	**0.034**
Monocyte infiltration			
Antrum	1.8 ± 0.4	1.9 ± 0.3	0.500
Corpus	2.2 ± 0.4	1.9 ± 0.5	0.188
AG			
Antrum	1.3 ± 0.7	0.6 ± 0.8	0.102
Corpus	0.9 ± 0.8	0.4 ± 0.7	0.273
IM			
Antrum	1.0 ± 0.8	0.4 ± 0.7	0.113
Corpus	0.6 ± 0.8	0.1 ± 0.3	0.098
PG I/II ratio	2.0 ± 0.7	5.6 ± 4.3	**0.022**

Adapted from Shin et al. [5]

p-values were calculated using the Chi-square test or Student's t-test

[a] Histologic grade ≥ 1 atrophic gastritis or intestinal metaplasia and pepsinogen I/II ratio < 2.5. *AG* atrophic gastritis, *IM* intestinal metaplasia, *PG* pepsinogen

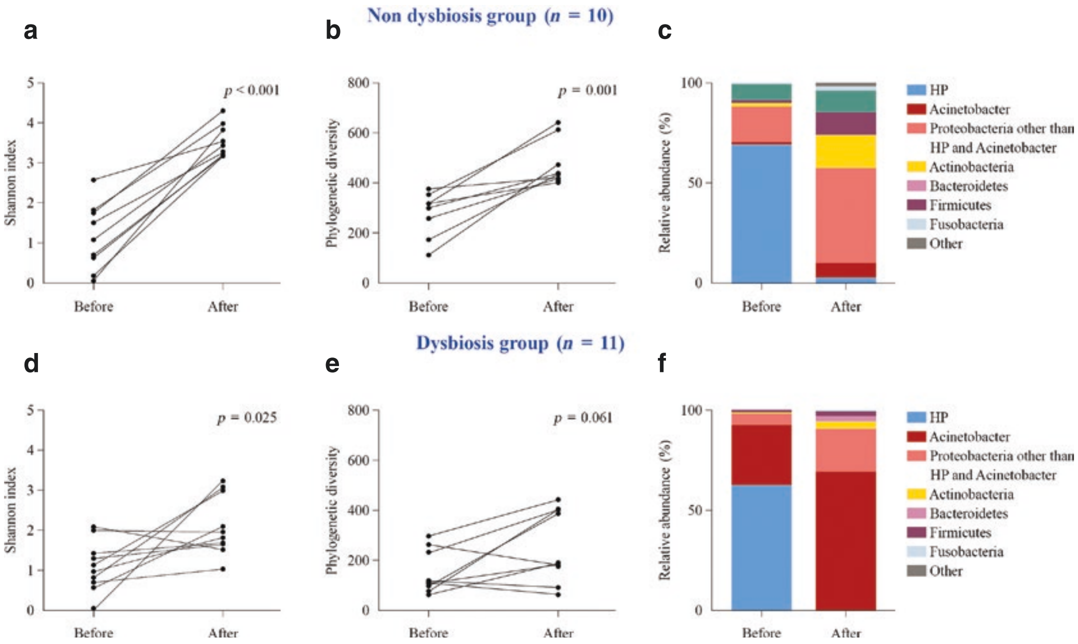


Fig. 60.7 Changes in gastric microbiota after *H. pylori* eradication. In the eradicated without dysbiosis group ($n = 10$), *H. pylori* eradication led to a restoration of diverse gastric microbiota composition (**a**–**c**). However, in the eradicated with dysbiosis group ($n = 11$), severe dysbiosis with an increase in *Acinetobacter* species abundance and a decrease in microbial diversity was observed (**d**–**f**) (Adapted from Shin et al. [5])

60.3.3 The Effect of *H. pylori* Eradication on the Gastric Microbiota Function

Three studies that performed bioinformatic analyzes evaluated changes of gastric microbiota functions after *H. pylori* eradication [7, 16, 17]. These studies yielded different findings for microbial functions. That is, the bacteria reproduction-related pathways down-regulated, such as bacterial chemotaxis [7, 16], flagellar assembly [7, 16], NOD-like receptor signaling [17], etc.; and normal gastric function-related pathways up-regulated, such as gastric acid secretion [7], protein digestion and absorption [7], and amino acid metabolism [17]. Further studies in depth are needed.

60.4 The Effect of *H. pylori* Eradication on the Gut Microbiota

Antibiotics used for *H. pylori* eradication can alter the gut microbiota and it is analyzed by NGS with stool samples instead of gastric mucosa. There are controversials so far. For instance, the gut microbiota was affected by *H. pylori* eradication regimens temporarily [23]. However, another study has reported significant changes in the gut microbiota structure and an increase in antibiotic resistance by quadruple *H. pylori* eradication therapy [8]. However, other studies showed that gut microbiota changes caused by antibiotic treatment were reversible and the restoration of gut microbiota a few weeks after *H. pylori* eradication [8, 16].

60.4.1 The Effect of *H. pylori* Eradication on the Gut Microbiota Depending on *H. pylori* Eradication Regimens

Taiwanese study compared the α- and β-diversity of gut microbiota among three *H. pylori* eradication regimens at week 2, 8, and 1 year: first triple therapy for 14 days (T14; lansoprazole 30 mg, amoxicillin 1 g, and clarithromycin 500 mg, all given twice daily), second concomitant therapy for 10 days (C10; lansoprazole 30 mg, amoxicillin 1 g, clarithromycin 500 mg, and metronidazole 500 mg, all given twice daily), and third bismuth quadruple therapy for 10 days (BQ10; bismuth tripotassium dicitrate 300 mg four times a day, lansoprazole 30 mg twice daily, tetracycline 500 mg four times a day, and metronidazole 500 mg three times a day) [8]. They found that α-diversity was significantly reduced 2 weeks after T14 ($p = 0.0002$), C10 ($p < 0.0001$), and BQ10 ($p < 0.0001$) treatment. β-diversity was also significantly altered 2 weeks after T14 ($p = 0.0010$), C10 ($p = 0.0001$), and BQ10 ($p = 0.0001$). These decreased α- and β-diversity was restored at week 8 ($p = 0{\cdot}14$ and $p = 0.918$, respectively) and 1 year ($p = 0.14$ and $p = 0.918$) after T14, but were not fully recovered at week 8 and after 1 year in patients treated with C10 ($p = 0.0001$ and $p = 0.013$ at week 8; $p = 0.019$ and $p = 0.064$ at 1 year) and BQ10 ($p < 0.0001$ and $p = 0.0002$; $p = 0.001$ and $p = 0.029$) [8]. This study evaluated the antibiotic resistance in gut microbiota after these three *H. pylori* eradication regimens. They found that a transient increase at week 2 after T14 and C10 of the resistance rates of *E. coli* to ampicillin-sulbactam (12% [15/127] to 66% [38/58] for T14, 7% [10/135] to 64% [28/44] for C10), cefazolin (13% [16/127] to 43% [25/58] for T14, 10% [13/135] to 41% [18/44] for C10), cefmetazole (8% [10/127] to 26% [15/58] for T14, 4% [5/135] to 18% [8/44] for C10), levofloxacin (8% [10/127] to 35% [20/58] for T14, 7% [10/135] to 32% [14/44] for C10), gentamicin (13% [19/146] to 47% [27/58] for T14, 15% [22/149] to 45% [20/44] for C10), and trimethoprim–sulfamethoxazole (33% [48/146] to 86% [50/58] for T14, 28% [42/148] to 86% [38/44] for C10; all $p < 0.05$ in paired samples in the above analyzes) returned to basal state at week 8 and after 1 year [8]. These results suggest that antibiotic resistance is not crucial in the choice of *H. pylori* eradication regimens.

60.4.2 The Effect of Probiotics on the *H. pylori* Eradication-Induced Gut Microbiota Changes

South Korean team performed 16S rRNA gene-pyrosequencing in stool before and after standard triple therapy using 500 mg clarithromycin, 1 g amoxicillin, and 30 mg of lansoprazole, twice daily for 2 weeks with or without combination with probiotics [10]. Before treatment, three phyla, Firmicutes, Bacteroidetes, and Proteobacteria, were predominant in the gut microbiota of all subjects [10]. After treatment of standard triple therapy for 2 weeks, the relative abundances of Firmicutes were reduced, whereas those of Proteobacteria were increased in both groups. Before the subjects were treated, the average number of the observed OUTs in the fecal samples obtained from the antibiotics group (219.2) was similar to that of the probiotics group (231.0). However, the numbers of operational taxonomy units (OTUs) of the antibiotics alone group (120.3) was lower than those of the probiotics group (186.8) after treatment (Fig. 60.8a). These differences were also found in the Shannon diversity indices (Fig. 60.8b). The changes in the microbial diversity were also detected in the rarefaction curves. The number of observed OTUs in the antibiotics alone group after treatment

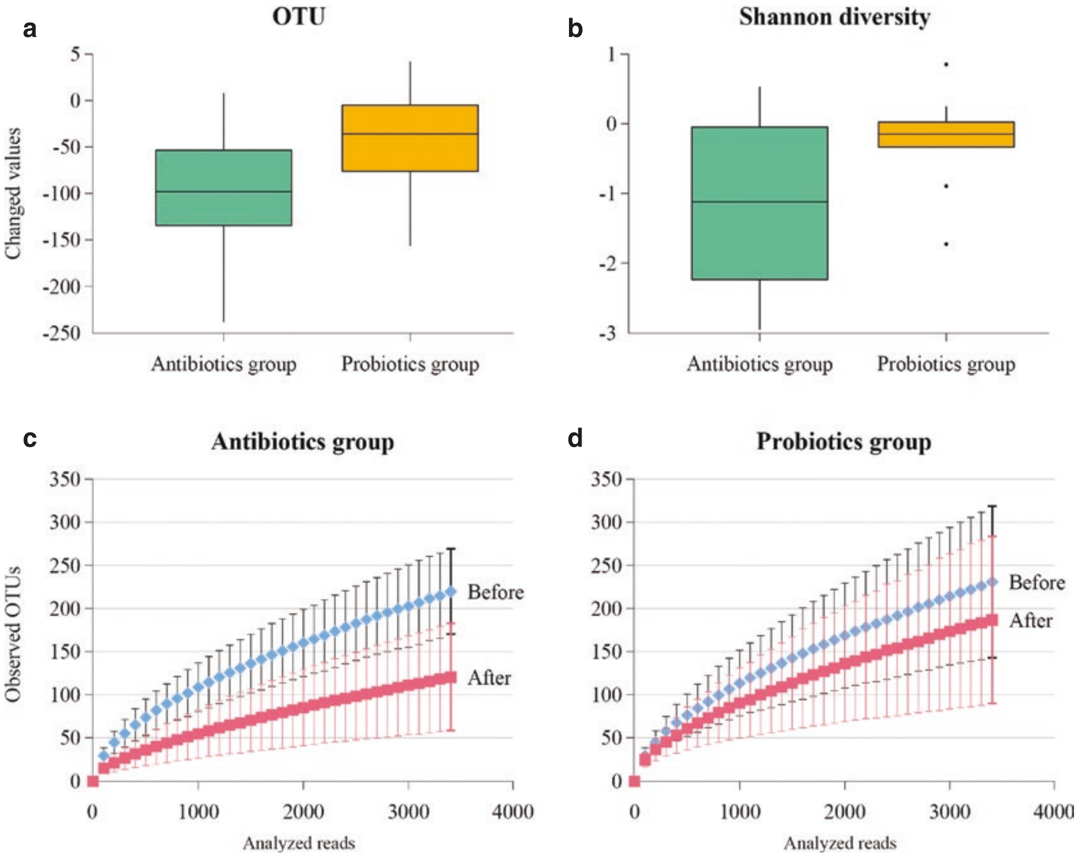


Fig. 60.8 The comparison of the diversity indices between the two groups. (**a**) The numbers of observed OTUs were compared between the two groups using box-plot analysis. (**b**) Shannon diversity indices were compared. (**c**) Rarefaction curves obtained from the antibiotic alone treated group were compared between before and after treatment. (**d**) Rarefaction curves of the probiotics co-treated group were compared between before and after treatment. *OTUs* operational taxonomy units (Adapted from Oh et al. [10])

(Fig. 60.8c) was decreased compared to the probiotics group (Fig. 60.8d). The authors suggested that probiotic supplementation can reduce the antibiotic-induced alteration and imbalance of the gut microbiota composition [10].

60.4.3 The Effect of Probiotics on the *H. pylori* Eradication-Induced Gut Microbiota Function

These authors further examined the impact of probiotic supplementation on the functional dynamics of the gut microbiota using KEGG Orthologue (KO) categories during *H. pylori* eradication, using whole-metagenomic sequence analysis [24]. After 2-week standard triple eradication there was change of relative abundance of KO categories (Fig. 60.9a). Interestingly, the proportion of genes related to metabolism was reduced after *H. pylori* eradication therapy in the probiotic supplemented group, whereas genes associated with human diseases increased in the antibiotic group [24] (Fig. 60.9b).

In particular, the proportion of genes associated with the metabolism of amino sugar and nucleotide sugars increased (Fig. 60.10a) whereas selenocompound metabolism was reduced in the probiotics group [24] (Fig. 60.10b). These results suggest that probiotic supplementation reduce the antibiotic-induced intestinal irritation and it helps the maintenance of bacterial diversity [24].

60.4.4 Gastric Microbial Composition Changes in the Persistent *H. pylori* Infection

When changes in microbial diversity and bacterial composition were evaluated in patients who failed to eradicate *H. pylori* or who did not

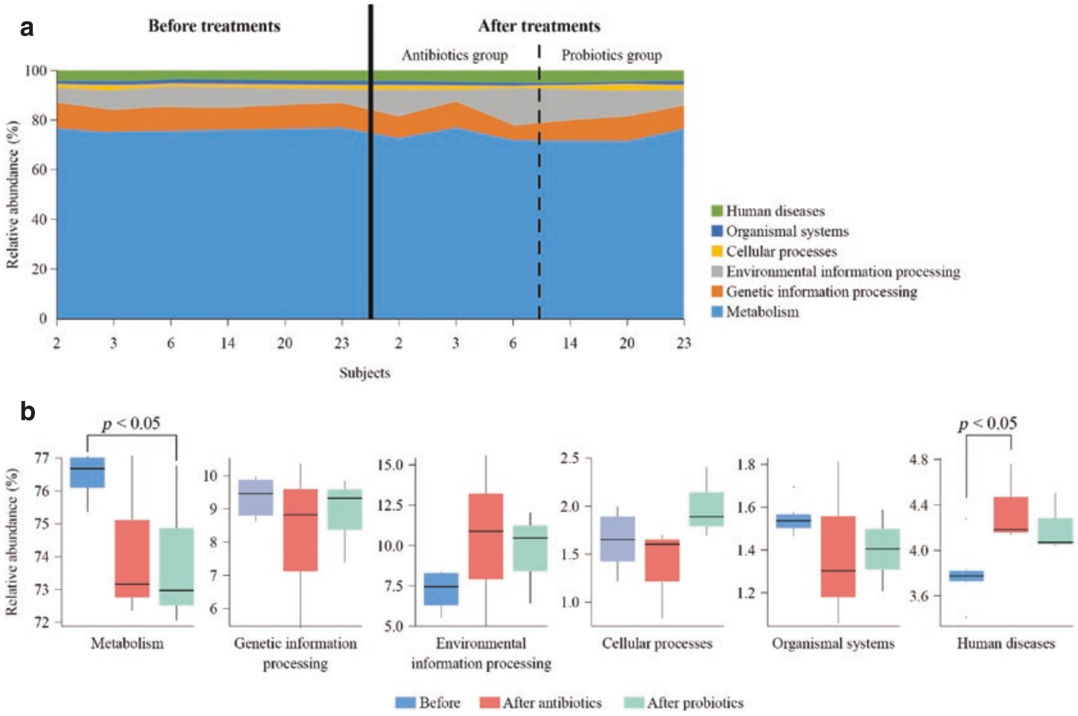


Fig. 60.9 The relative abundance of KEGG Orthologue (KO) categories among the subjects compared using area charts. (**a**) The relative abundance was compared before and after standard triple *H. pylori* eradication therapy. (**b**) The proportion of genes related to metabolism was reduced in the probiotic supplemented group, whereas genes associated with human diseases increased in the antibiotic group (Adapted from Oh et al. [26])

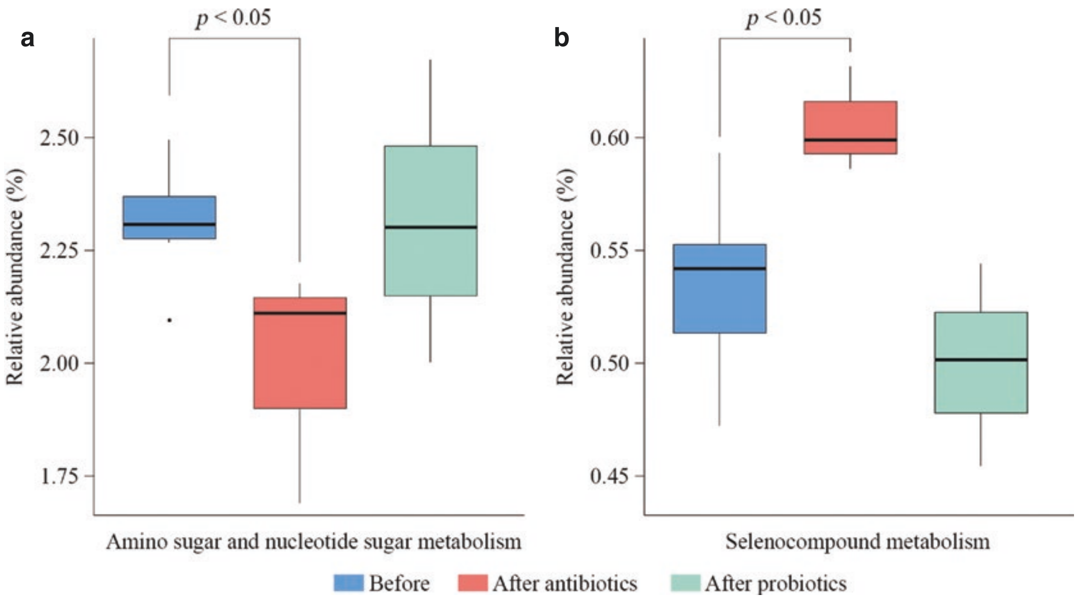


Fig. 60.10 The median values of similarly altered categories in both groups were presented as box plots ($p < 0.05$). The bacterial species associated with each category of metabolism were compared between groups. The changes in the proportion of genes in the amino sugar and nucleotide sugar metabolism (**a**), as well as selenocompound metabolism (**b**), were decreased in antibiotics groups but they were returned to the levels of pretreatment group with probiotics (Adapted from Oh et al. [24])

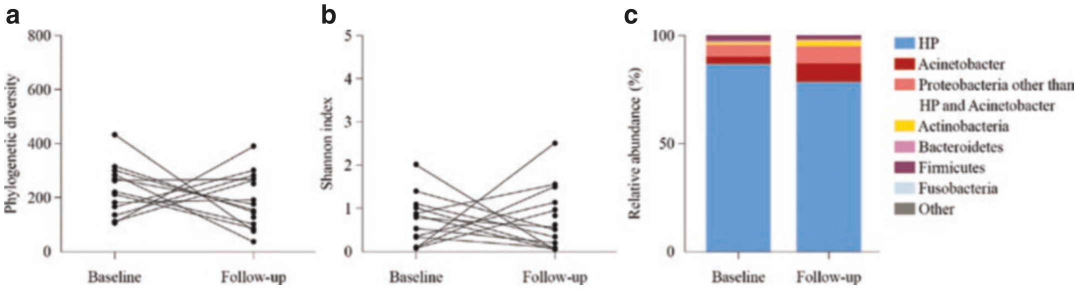


Fig. 60.11 No changes of α-diversity indices and bacterial composition in gastric microbiota in subjects with persistent *H. pylori* infection (**a**) Phylogenetic diversity, (**b**) Shannon index, and (**c**) bacterial composition were not changed significantly (Adapted from Shin et al. [5])

receive anti-*Helicobacter* treatment (n = 13), there was no significant change in microbial diversity and bacterial composition at the phylum level during follow-up in most patients with persistent infection [5] (Fig. 60.11).

60.5 Conclusions

Chronic infection of *H. pylori* causes predominant occupation of *H. pylori* resulting in microbial dysbiosis of gastric microbiota. This dysbiosis can contribute to the gastric carcinogenesis. Thus, many guidelines recommend *H. pylori* eradication to prevent the GC development. Researchers have performed studies regarding the gastric microbiota changes after *H. pylori* eradication by NGS with gastric mucosa. The results remain controversial whether successful *H. pylori* eradication could restore gastric microbiota to uninfected status. α-diversity indexes increased within 1 month after *H. pylori* eradication and significant differences in gastric microbial community structure between before and after *H. pylori* eradication were also seen within 1 month. However, our group found that an increase in *Acinetobacter* species abundance was associated with persistent gastric inflammation, and negatively correlated with the histologic grade of atrophy and IM after successful *H. pylori* eradication. These findings suggest that gastric dysbiosis after *H. pylori* eradication is not temporary but could persist for years. Regarding the issue of antibiotics associated changes of gut microbiota outside of the stomach, they were reversible and the restoration of gut microbiota a few weeks after *H. pylori* eradication has been reported. In conclusion, *H. pylori* eradication causes beneficial change in the gastric microbiota with minimal disruption of the gut microbiota. Probiotic supplementation reduced this minimal disruption of the gut microbiota.

References

1. Cover TL, Blaser MJ. *Helicobacter pylori* in health and disease. Gastroenterology. 2009;136:1863–73.
2. Andersson AF, Lindberg M, Jakobsson H, Bäckhed F, Nyrén P, Engstrand L, et al. Comparative analysis of human gut microbiota by barcoded pyrosequencing. PLoS One. 2008;3:e2836.
3. Bik EM, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, et al. Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci U S A. 2006;103:732–7.
4. Li TH, Qin Y, Sham PC, Lau KS, Chu KM, Leung WK. Alterations in gastric microbiota after *H. pylori* eradication and in different histological stages of gastric carcinogenesis. Sci Rep. 2017;7:44935.
5. Shin CM, Kim N, Park JH, Lee DH. Changes in gastric corpus microbiota with age and after *Helicobacter pylori* eradication: a long-term follow-up study. Front Microbiol. 2021;11:621879.
6. Guo Y, Cao XS, Guo GY, Zhou MG, Yu B. Effect of *Helicobacter pylori* eradication on human gastric microbiota: a systematic review and meta-analysis. Front Cell Infect Microbiol. 2022;12:899248.
7. Guo Y, Zhang Y, Gerhard M, Gao JJ, Mejias-Luque R, Zhang L, et al. Effect of *Helicobacter pylori* on gastrointestinal microbiota: a population-based study in Linqu, a high-risk area of gastric cancer. Gut. 2020;69:1598–607.
8. Liou JM, Chen CC, Chang CM, Fang YJ, Bair MJ, Chen PY, et al. Long-term changes of gut microbiota, antibiotic resistance, and metabolic parameters after *Helicobacter pylori* eradication: a multicentre, open-label, randomised trial. Lancet Infect Dis. 2019;19:1109–20.
9. Cornejo-Pareja I, Martín-Núñez GM, Roca-Rodríguez MM, Cardona F, Coin-Aragüez L, Sánchez-Alcoholado L, et al. *H. pylori* eradication treatment alters gut microbiota and GLP-1 secretion in humans. J Clin Med. 2019;8:451.
10. Oh B, Kim BS, Kim JW, Kim JS, Koh SJ, Kim BG, et al. The effect of probiotics on gut microbiota during the *Helicobacter pylori* eradication: randomized controlled trial. Helicobacter. 2015;21:165–74.
11. Kang JM, Kim N, Kim JH, Oh E, Lee BY, Lee BH, et al. Effect of aging on gastric mucosal defense mechanisms: ROS, apoptosis, angiogenesis, and sensory neurons. Am J Physiol Gastrointest Liver Physiol. 2010;299:G1147–53.
12. Jo HJ, Kim N, Nam RH, Chang H, Kim JH, Park JH, et al. The effect of *Cochinchina momordica* seed extract on gastric acid secretion and morphologic change in aged rat stomach. Gut Liver. 2013;7:560–8.
13. Ferreira RM, Pereira-Marques J, Pinto-Ribeiro I, Costa JL, Carneiro F, Machado JC, et al. Gastric microbial community profiling reveals a dysbiotic cancer-associated microbiota. Gut. 2018;67:226–36.
14. Mao LQ, Zhou YL, Wang SS, Chen L, Hu Y, Yu LM, et al. Impact of *Helicobacter pylori* eradication on the gastric microbiome. Gut Pathog. 2021;13:60.
15. Serrano CA, Pierre R, Van Der Pol WJ, Morrow CD, Smith PD, Harris PR. Eradication of *Helicobacter pylori* in children restores the structure of the gastric bacterial community to that of noninfected children. Gastroenterology. 2019;157:1673–5.
16. He C, Peng C, Wang H, Ouyang Y, Zhu Z, Shu X, et al. The eradication of *Helicobacter pylori* restores rather than disturbs the gastrointestinal microbiota in asymptomatic young adults. Helicobacter. 2019;24:e12590.
17. Sung JJY, Coker OO, Chu E, Szeto CH, Luk STY, Lau HCH, et al. Gastric microbes associated with gastric inflammation, atrophy and intestinal metaplasia 1 year after *Helicobacter pylori* eradication. Gut. 2020;69:1572–80.
18. Watanabe T, Nadatani Y, Suda W, Higashimori A, Otani K, Fukunaga S, et al. Long-term persistence of gastric dysbiosis after eradication of *Helicobacter*

pylori in patients who underwent endoscopic submucosal dissection for early gastric cancer. Gastric Cancer. 2021;24:710–20.

19. Yuan Z, Xiao S, Li S, Suo B, Wang Y, Meng L, et al. The impact of *Helicobacter pylori* infection, eradication therapy, and probiotics intervention on gastric microbiota in young adults. Helicobacter. 2021;26:e12848.
20. Enjalbert L, Brisou J, Kambou AK. Isolation of an urease-positive Acinetobacter group in man. Ann Inst Pasteur (Paris). 1959;97:112–5.
21. Doughari HJ, Ndakidemi PA, Human IS, Benade S. The ecology, biology and pathogenesis of Acinetobacter spp.: an overview. Microbes Environ. 2011;26:101–12.
22. Munoz-Price LS, Weinstein RA. Acinetobacter infection. N Engl J Med. 2008;358:1271–81.
23. Iizumi T, Battaglia T, Ruiz V, Perez Perez GI. Gut microbiome and antibiotics. Arch Med Res. 2017;48:727–34.
24. Oh B, Kim BS, Kim BS. Changes in the functional potential of the gut microbiome following probiotic supplementation during *Helicobacter pylori* treatment. Helicobacter. 2016;21:493–503.

Part X

Animal Model

61

Animal Models of *H. pylori* Infection

Ju Yup Lee

Abstract

The best suited animal models of *Helicobacter pylori* (*H. pylori*) identified so far are rodents. *Helicobacter felis* infection provokes severe inflammation and induces well sequential carcinogenic events leading to dysplasia and gastric adenocarcinoma. *H. pylori* SS1 is the most useful strain identified so far. It expresses cytotoxin-associated gene A (*cagA*) and vacuolating cytotoxin (*vacA*) and can easily colonize the stomach of mice and induce humanlike gastric pathological changes. However, a long-term colonization of the stomach does not always induce dysplasia or adenocarcinoma. Unlike *H. pylori* SS1, however, it lacks the *cag* pathogenicity island (PAI) and does not adhere well to gastric epithelial cells. Individuals colonized by the same strain show different responses. This is ascribable to T cell immune responses, whereby the balance between the immune responses of Th1 and Th2 plays an important role. A variety of transgenic or knockout mice have been developed and used. Such animal models have greatly contributed to identifying immunophysiology associated with *H. pylori*.

Keywords

Animal · *Helicobacter felis* · *Helicobacter pylori* SS1

61.1 Introduction

For the past decades, animal infection models have been efficiently used for identifying the pathophysiology of *Helicobacter pylori* (*H. pylori*)-related diseases. The following summarizes the achievements made to date using *H. pylori* animal models [1]. First, gastric acid has been found to play an important role in the creation of favorable habitat conditions for *H. pylori*. This explains why different population groups tend to develop different diseases and why gastric ulcers affect specific regions. Second, studies with different mouse species have led to the finding that individual factors play an important role in the colonization process. Third, pathophysiologic mechanisms of a variety of bacterial virulence factors, such as urease, have been identified. Fourth, the immuno-pathophysiological mechanisms have been identified through the knockout mice, and vaccination against *H. pylori* is possible. Lastly, antimicrobial research has gained momentum.

The foci of this chapter are the representative strains used for the animal models of *H. pylori* infection, animal species, predominantly rodents that are most commonly used at present and a variety of transgenic or knockout mice.

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61.2 Selection of Animal Models

H. pylori is well adapted for colonizing the human stomach, but the natural history of infection in animals is unknown, and it does not easily infect the gastric mucosa of animals. To date, many different animal species have been used in *H. pylori* infection research, ranging from primates to rodents. Big animals, such as monkeys, pigs, and dogs, were mostly used in earlier phases of research using animal models. Such models have been found to be easily colonized by *Helicobacter* strains [2–4]. While they have gastric physiology similar to that of humans, they are usually costly and have low values for practical use. Moreover, while lymphocytic gastritis could be induced in these animal models, severer conditions than that, such as gastric ulcer or adenocarcinoma, did not occur. Above all, their major disadvantage is the limitation in long-term utilization and the colonization of endemic *Helicobacter* strains, such as *H. heilmannii* for primates and *H. suis* for pigs [5].

Small animals such as ferrets, Mongolian gerbils, rats, and mice are more convenient to handle than big animals and have many additional advantages. The first report of carcinogenesis by *Helicobacter* strains was made on ferret models, whereby gastritis, dysplasia, and gastric adenocarcinoma were induced in ferret stomachs colonized by *H. mustelae* [6]. Moreover, ferrets are the first animal model in which gastric ulcers were verified [7]. However, ferrets are not easily available and the strain used for them, *H. mustelae*, has different properties compared to *H. pylori* that colonizes the stomach of humans.

Mongolian gerbils are easily colonized by *H. pylori* and once they are infected, they show the sequential steps of chronic active gastritis, peptic ulcers, intestinal metaplasia, and gastric cancer similar to those of humans [8]. Therefore, Mongolian gerbils work as a suitable animal model and are the most commonly used animal model for establishing *H. pylori* infection. G1.1, TN2, and B128 are some of the *H. pylori* strains that were verified to induce gastric adenocarcinoma by colonizing Mongolian gerbils. Additionally, in relation to many reports on the increased incidences of gastric cancer in the Mongolian gerbil group administered first chemical gastric carcinogenic agents such as MNU (*N*-methyl-*N*-nitrosourea) and MNNG (*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine) and additionally infected with *H. pylori* when compared to the MNU or MNNG single administration group, there are also views that *H. pylori* plays the catalytic role for the gastric carcinogenesis in Mongolian gerbils rather than being single carcinogenic factor [9, 10]. While Mongolian gerbils are excellent as an animal model of *H. pylori* infection and induce inflammatory responses similar to humans, they are costly and lack transgenic or knockout species and thus compare unfavorably to mice in terms of practical use.

A mouse model infected with the *H. pylori* Sydney strain 1 (SS1) displayed chronic gastritis and gastric atrophy [11]. However, wild-type mouse models, such as C57BL/6 [12], BALB/c [13], and C3H [14], infected with *H. pylori* commonly develop mild gastritis or slow progressing diseases and provide less information about *H. pylori* pathogenicity [15–17]. Infected mice models with *H. pylori* and *H. felis* resulted in lymphocytic gastritis without progression to severe pathologies, such as peptic ulcers or gastric cancer [18, 19]. Moreover, the architecture of the murine stomach differs from that of the human stomach and lacks the components necessary for the development of severe gastric pathologies. Furthermore, the murine stomach may contain other bacteria that may influence the outcome of *H. pylori* infection [18, 19].

The guinea pig model of *H. pylori* infection was first described in the late 1990s [20]. The guinea pig is a small laboratory animal with a stomach structure similar to that of humans, prone to developing an inflammatory response due to IL-8 secretion by gastric epithelial cells. Similar to the mouse model, the guinea pig models exhibit the ease of husbandry owing to the

Table 61.1 Advantages and disadvantages of *Helicobacter*-infected animal models

Animal	Colonized by	Advantage	Disadvantage
Primate	*H. pylori*	1. Gastric physiology of primate models is very similar to humans 2. Endoscopy is possible due to large body size	1. Expensive 2. Colonized by endemic strains of *Helicobacter heilmanniilike* bacteria (HHLO)
Gnotobiotic piglet	*H. pylori*	1. Shows similar colonization pattern to human 2. Infection leads to gastric ulceration, similar to human	1. Expensive 2. Can be used for short-term experiments only 3. Shows chronic gastritis only
Ferret	*H. mustelae*	1. Infected from nature, useful for vaccine development 2. Gastric physiology is almost similar to human	1. Colonization pattern differs from human 2. Disease pathology is limited to chronic gastritis only
Cat and dog	*H. pylori* *H. felis*	1. Gastric physiology is almost similar to human	1. Gnotobiotic and specific pathogen-free (SPF) animals are expensive 2. Normal cats harbor HHLO in the gastric mucosa
Guinea pig	*H. pylori*	1. Overt secretion of IL-8 homolog 2. Shows active chronic gastritis	1. Available reports are not conclusive enough
Mouse	*H. pylori* *H. felis* *H. heilmannii*	1. Economically feasible 2. An important model for testing the efficacy of vaccine/antimicrobials	1. Gastric physiology of mice does not exactly reflect the human condition
Mongolian Gerbil	*H. pylori*	1. Gerbil models, upon infection, show chronic/active antral gastritis, leading to gastric ulcers and adenocarcinoma	1. Transgenic/knockout strains are unavailable. 2. Gerbil-specific antibodies are not easily available

Adapted from Lee [1]

SPF specific pathogen free; *IL-8* interleukin 8; *HHLO Helicobacter heilmannii*-like bacteria

small animal size. In addition, the guinea pig stomach possesses several features in common with the human stomach, such as the presence of a cylindrical epithelium, maintenance of sterile conditions, the ability to produce IL-8, and the lack of a non-glandular region [21]. However, the studies are from 2001 to 2003, and there was no openness to metagenomics studies that show the opposite in humans [22].

Tables 61.1 and 61.2 outline the advantages and disadvantages about the animal models developed thus far [1, 4]. The conditions for an ideal animal model are cost-effectiveness, high reproducibility, short observation period, and pathogenesis leading to lesions close to human histological changes, and up to now, rodents have been found to meet these conditions better than any other animals [8]. Mice, in particular, have additional advantage of easy genetic manipulation and are being used most widely for *H. pylori* research.

Table 61.2 Factors to consider in selecting *Helicobacter*-infected animal models

	Most	Favorable	Least
Cost and availability	Mice-rats	Ferrets/cats	Pigs, primates
Reproduces human disease	Primates-pigs-gerbils-ferrets		Mouse models
Availability of reagents	Mice-primates		Ferrets-gerbils

Adapted from Nedrud [4].

61.3 Selection of Strains

With mice showing resistance to the colonization of various *H. pylori* strains, research for developing suitable strains for mouse models has been underway. Of the strains hitherto identified, *H. felis* and *H. pylori* SS1 are known to be the most promising strains [23].

61.3.1 Characteristic and Morphologic Differences Between *H. felis* and *H. pylori* SS1

H. felis is a *Helicobacter* species isolated from cat gastric mucosa. It has a structure very similar to that of *H. pylori* and grows well in the stomach of mice. In the 1990s, Lee et al. [24] made the first report on acute and chronic gastritis in germ-free Swiss Webster mice infected with *H. felis* similar to inflammatory responses in humans. *H. felis* was also the first *Helicobacter* species used in the adoptive transfer mouse model to demonstrate the importance of T helper cells (Th1) in disease [25]. In subsequent studies, numerous *H. felis* colonized the stomach of mice in large numbers, inducing severe gastritis and atrophic gastritis [26], and long-term colonization models developed intestinal metaplasia, dysplasia, and invasive gastric cancer over time [27, 28]. However, different from *H. pylori* colonizing human gastric mucosa, *H. felis* shows noticeably different pathological features from the pathophysiological changes occurring in human gastric mucosa [8]. Moreover, *H. felis* lacks the cytotoxin-associated gene pathogenicity island (*cag* PAI) and does not adhere well to gastric epithelial cells, floating in the gastric mucosa (Table 61.3). Therefore, using *H. felis* for animal models would serve the purpose in studies investigating host factors, but may have limitations in studies investigating virulence factors [29].

In an attempt to overcome these limitations, many studies have focused on *Helicobacter* strains that can grow well on the gastric mucosa of mice and have many virulence factors. In 1997, Lee et al. [11] succeeded in isolating *H. pylori* in the gastric mucosa of a 42-year-old who had suffered from gastric ulcer for 10 years and developed an animal model using this strain, which they named *H. pylori* SS1. Known to be the most useful strain identified so far, it expresses *cagA* and vacuolating cytotoxin (*vacA*), easily infects mice, and is reported to induce pathological changes similar to human gastric diseases [11]. Unlike *H. felis* that has weak adhesion or looks as if floating in the mucus when observed with an electron microscope, *H. pylori* SS1 is characterized by tight adhesion to the epithelial cells via fine projections with adherence pedestals [11] (Table 61.3). Another particular feature of *H. pylori* SS1 is that it colonizes BALB/c, DBA/2, and C3H/He mice in lower numbers while developing colonies in large numbers in C57BL/6 mice [11, 23].

Table 61.3 Differences between *H. felis* and *H. pylori* SS1

H. felis	*H. pylori* SS1
Isolated from cat gastric mucosa	Isolated from human gastric mucosa
Lacks the *cag* pathogenicity island (PAI)	*cagA*(+), *vacA*(+)
Colonizes the mucus layer	Adheres directly to epithelial cells
Chronic active gastritis progressing to severe hyperplasia, atrophy, dysplasia, and carcinoma	Chronic active gastritis progressing to severe hyperplasia and atrophy
	Rarely induced dysplasia or carcinoma

In 2002, a controversy arose regarding the use of *H. pylori* SS1 in a mouse disease model. This was based on the finding that while *H. pylori* SS1 contained the *cag* PAI, it did not appear to have a functional type IV secretion system (T4SS) as determined in cell culture [30, 31]. To address this limitation, investigators have used pre-mouse SS1 (PMSS1), the original human isolate that has a functional T4SS and gave rise to *H. pylori* SS1 after mouse passage [32].

In 2004, another strain (Sydney strain 2000, SS2000) that has virulence factors suitable for mice was isolated by Thompson et al., and this strain has been used for various virulence-related research models [33]. On the other hand, *H. pylori* ATCC43504 strain reported by Hirayama et al. [34] in Japan in 1996 showed 100% infection rate when Mongolian gerbils were colonized and induced gastritis pathologically similar to human gastritis. Along with *H. pylori* SS1, it is being widely used for various mouse models.

61.3.2 Pathological Difference Between *H. felis* and *H. pylori* SS1

Unlike *H. pylori*-related gastritis in humans, in which both neutrophils and monocytes are deposited on mucous membranes [35], neutrophils are less deposited in mouse models, as observed in both *H. felis* and *H. pylori* infections. While a chronic colonization of the mouse stomach by *H. felis* and *H. pylori* SS1 leads to chronic gastritis and premalignant lesions, there is an important difference between the two. Almost all *H. felis* infections in the C57BL/6 mice develop into gastric adenocarcinoma via sequential steps from intestinal metaplasia and dysplasia [36, 37], which is very similar to the human gastric carcinogenesis. In contrast, *H. pylori* SS1 failed to induce gastric adenocarcinoma in most mouse species [23]. Kim et al. [38] observed *H. pylori* SS1-infected C57BL/6 mice for 80 weeks and reported that while hyperplastic gastritis or chronic atrophic gastritis was easily induced, no incidence of dysplasia or gastric adenocarcinoma was observed and explained the reason to be the balance between cell proliferation and apoptosis. *H. felis* infection in the C57BL/6 mouse induces severe inflammation whereby the degree of early-phase inflammation shows higher level compared to *H. pylori* SS1 infection. Lee et al. [39] observed *H. pylori* SS1-infected C57BL/6 mice for 4 weeks and verified that inflammatory responses and hyperplastic gastritis are induced well. The same mice were then infected with *H. felis* and observed for 4, 24, and 52 weeks. They showed severer inflammatory responses, with significantly higher level of myeloperoxidase, a marker of inflammation, during the same period of 4 weeks compared to the *H. pylori* SS1 infection group [40]. Furthermore, from the fact *H. felis* infection led to significant increase in interleukin (IL)-1β and no increase in tumor necrosis factor (TNF)-α, it can be inferred that IL-1β is involved in the immunological mechanism of the infected host [40]. Accordingly, the different characters of *H. pylori* and *H. felis* strains should be considered when establishing animal models of *Helicobacter* infection.

61.4 Differences in Individual Responses

Individual genetic factors play an important role in inflammatory responses or carcinogenesis of individual animals. When a mouse species was infected with a *Helicobacter* species, each mouse showed noticeably different responses. For example, histological changes observed 1, 2, and 6 months after infecting different mice with the same *Helicobacter* species *H. felis* yielded different results in different mouse models. While SJL, C3H/He, DBA/2, and C57BL/6 mice developed moderate-to-severe chronic active gastritis in the corpus, which got exacerbated over time, resulting in atrophy, BALB/c and CBA mice showed only mild antral gastritis, and no change was observed in the corpus, nor did atrophy occur [26]. Based on these results, it was proposed to classify the animal models into responder strains and nonresponder strains depending on the degree of individual inflammatory responses after *H. pylori* infection [26]. According to this classification, C57BL/6 and C3H/He can be defined as responder strains and BALB/c and CBA as nonresponder strains. However, when the nonresponder strain BALB/c mouse underwent long-term infection with *H. felis*, mucosa-associated lymphoid tissue (MALT) lymphoma-like pathology was observed [41]. In other words, as a result of observing BALB/c mice during 22 months of *H. felis* infection, it was found that 38% BALB/c mice developed MALT lymphoma, whereas the uninfected control group did not develop it [41].

The interindividual differences in inflammatory responses among the mice infected with the same strain can be explained by the differences in T cells. RAG-1$^{-/-}$ mice, which lack both B and T cells, and TCRβδ$^{-/-}$ mice lacking T cells only did not show pathological abnormalities despite a high degree of *H. felis* colonization. In contrast, the B-cell-deficient μMT mice infected with *H. felis* exhibited severe pathological changes in the stomach such as mucosal hyperplasia and intestinal metaplasia, which was similar to the normal-state immune responses of C57BL/6 mice [42]. T cells play the most important role in the immune responses to *H. pylori* infection [43], and the balance of Th1/Th2 immune responses are essential in the pathogenesis of *H. pylori*-associated disease and host defense mechanisms (Fig. 61.1). In other words, while the predominance of Th1 immune responses greatly contributes to tissue damage, Th2 immune responses are known to

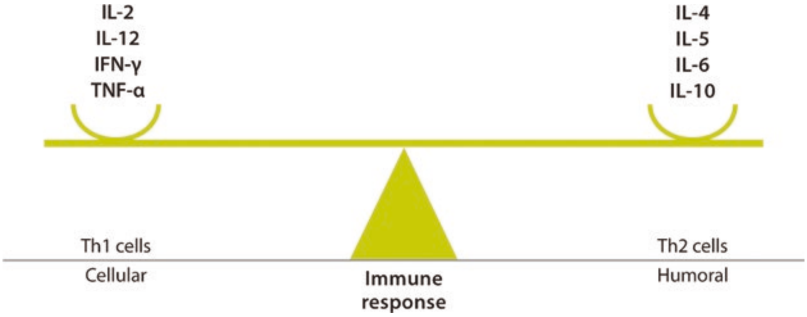

Fig. 61.1 Balance between Th1 (cell-mediated) and Th2 (humoral) immune responses. *IL* interleukin; *IFN-γ* interferon gamma; *Th1* T helper 1; *Th2* T helper 2

exhibit a defense mechanism against the inflammatory responses of the gastric mucosa [44]. C57BL/6 mice primarily show proinflammatory Th1-dominant immune responses, which leads to epithelial cell damage and compensatory hyperplasia even in a small number of colonies [45]. On the other hand, BALB/c mice primarily exhibit noninflammatory Th2-dominant immune response, which is characterized by a large number of colonies but a low proportion of epithelial lesions [33]. Various roles played by Th1 cytokine were proved by studies using gene-deficient mice. Among others, interferon (INF)-γ [46], TNF-α [47], and IL-7 [47] are known to be involved in the incidence of atrophic gastritis. Moreover, C57BL/6 mice with anti-inflammatory Th2 cytokine IL-10 deficiency developed severe hyperplastic gastritis even after short-term *H. felis* colonization (4 weeks) [48].

Furthermore, C57BL/6 mice are colonized by a large number of colonies in the antrum, whereas a small number of colonies were observed in BALB/c mice in the antrum-corpus transitional zone [11]. This reflects the fact that local gastric acid secretion is determinant for the position of *Helicobacter* strains within the stomach [49], which is ascribable to the interspecies differences in local gastric acid secretion or the difference in sensitivity to gastric acid of *H. pylori* or *H. felis* [11].

Moreover, phospholipase A_2 (PLA_2) was reduced in *H. felis*-infected C57BL/6 mice and increased in BALB/c mice according to research results [27, 50], whereas a high level of proinflammatory cytokine such as INF-γ was measured in C57BL/6 mice in a study. Studies with immunodeficient mouse models or mice with deficiency in cytokines such as IFN-γ, IL-10, IL-4, and IL-7 prove well the importance of the individual immune system in the occurrence of severe gastritis and accompanying changes in the mucous membrane [42, 47, 48, 51–56].

61.5 Transgenic or Knockout Mouse Models

Research efforts using a large variety of genetically manipulated mice have shed light on the immune mechanism of *Helicobacter* infection and the association of *H. pylori* with gastric cancer. Most genetically, manipulated mice are based on C57BL/6 mice. The following presents the representative mice used for clarifying pathophysiological mechanisms [45].

61.5.1 INS-GAS Mice

Transgenic INS-GAS mice overexpress human gastrin gene and thus develops spontaneous atrophic gastritis and gastric adenocarcinoma, and *Helicobacter* infection accelerates this process. The main contribution of this mouse model is the discovery of synergistic interaction between hypergastrinemia and *Helicobacter* infection in developing gastric adenocarcinoma [57].

61.5.2 IFN-γ and TNF-α Knockout Mice

From the observation of decrease in the degree of gastritis and change of epithelial cells when an IFN-γ-deficient mouse is infected with *H. felis* or *H. pylori*, it was found that IFN-γ plays an important role in the occurrence of *Helicobacter*-associated gastritis and epithelial cell damage [46, 58]. There is no consensus on the mechanisms associated with TNF-α. Hasegawa et al. [58] reported that TNF-α contributed to the carcinogenicity of *H. felis*-associated gastritis, whereas Yamamoto et al. [59] reported that gastric inflammatory responses did not decrease even in the absence of TNF-α. Lee et al. [40] also reported that long-term *H. felis* infection (52 weeks) did not yield any significant increase in the TNF-α level. Further research appears necessary to clarify this aspect.

61.5.3 IL-1β Transgenic Mice

IL-1β is a proinflammatory cytokine inhibiting gastric acid secretion, involved in the occurrence of atrophic gastritis and gastric adenocarcinoma [60]. It contributes to developing gastric adenocarcinoma in synergistic interaction with *Helicobacter* infection [61].

61.5.4 IL-10 Knockout Mice

IL-10 is known to be an anti-inflammatory and immunoregulatory cytokine. In IL-10-deficient mice with short-term (4 weeks) *H. felis* infection, severe hyperplastic gastritis and epithelial cell proliferation could be observed [48]. This arose from the Th1 immune responses and is indicative of the inhibitory mechanism of IL-10 in regulating immunity and inflammation.

61.5.5 Fas Antigen Transgenic Mice

Fas is a transmembrane protein that belongs to the TNF family and induces apoptosis. Apoptosis of epithelial cells is one of the most important responses of gastric mucosal membrane to *H. pylori* infection [62]. The Fas antigen transgenic mouse model contributed to gaining the knowledge that the Fas-inducing signaling system plays a role of preventing *Helicobacter* infection-associated carcinogenicity of chronic gastritis by inducing apoptosis [63].

61.5.6 p27-Deficient Mice

The cyclin-dependent kinase inhibitor p27 is a tumor-inhibiting protein, and its loss is thus a common clinical feature associated with poor prognosis [64]. Additionally, p27-deficient mice easily developed tumors when exposed to environmental carcinogenic agents [65]. In a 60-week observational study, p27-deficient mice infected with *H. pylori* SS1 developed metaplasia, dysplasia, and gastric cancer [66].

61.5.7 *cagA*-Transgenic Mice

Since *H. felis* lacks *cagA*, the *H. pylori* virulence factor, infection research with rodent models is often useless in clarifying the function of *cagA*. Moreover, even though *cagA* exists in *H. pylori* SS1, it often shows functional insufficiency for *cagA* translocation. To overcome this problem, the *cagA*-transgenic mouse model was developed, and *cagA* expression was reported to increase in the mouse stomach, resulting in gastrointestinal cancer, although the frequency was low [67].

61.6 Limitations of Mouse Models of Infection

In general, lab animals are exposed to high levels of stress due to artificial inbred and growing conditions in restricted spaces, poor hygienic state, excessive temperature, humidity, and light changes, which can affect the experimental results and even yield completely different results in severe stress situations. In case of lab mice as well, it should be always taken into consideration that sex, diet, habitat, and genetic environment can affect inflammatory and genetic manipulation-related responses. Furthermore, concurrent infection with an enterohepatic *Helicobacter* species with the experimentally induced infection with a *Helicobacter* strain in a mouse model can exert adverse influence on the results [68, 69].

The following issues can be pointed out as the limitations of mouse models [23]. First, there are only a limited number of *H. pylori* strains (espe-

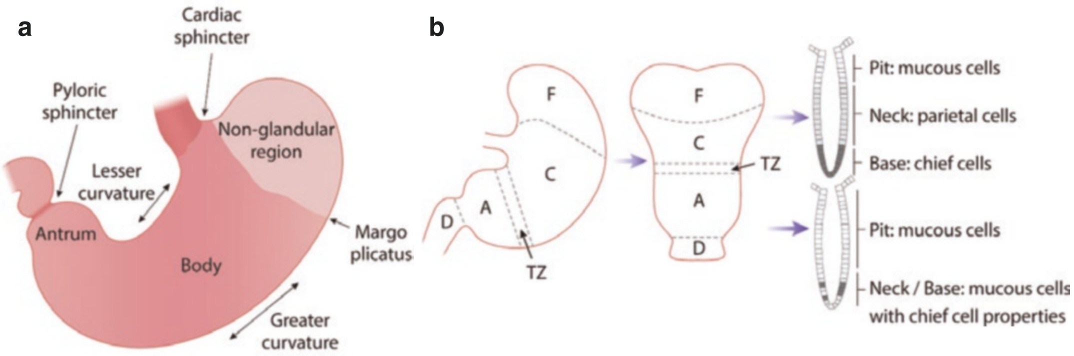

Fig. 61.2 Anatomical structure or mouse stomach. Mouse stomach is largely divided into glandular stomach, nonglandular forestomach, and the margo plicatus between these two parts. The glandular stomach and nonglandular forestomach consist of simple columnar epithelium containing the gastric gland and stratified squamous epithelium, respectively (**a**). The stomach corpus and antrum consist of different glandular cells and are separated by the corpus-antrum transition zone (**b**). *F* forestomach; *C* corpus; *TZ* transition zone; *A* antrum; *D* duodenum (Adapted from Ferrero et al. [70] and Rolig et al. [71])

cially *cagA*-positive strains) that can colonize the stomach of mice. Second, only long-term colonization induces tumors. Third, while high-severity dysplasia or early gastric cancer occurs frequently, the occurrence rate of progressive or invasive gastric cancer is very low and the occurrence rate of metastatic gastric cancer is zero. Fourth, there are anatomical differences between human and rodent stomachs. The mouse stomach can be largely divided into the nongrandular forestomach, glandular corpus, and antrum [70, 71] (Fig. 61.2). Although several reports have been made on forestomach squamous cell carcinoma in mouse models [72, 73], the human stomach does not have the forestomach, and gastric squamous cell carcinoma is a very rare disease in human. Thus, such reports have little usability. Additionally, no reports have yet been made on a mouse model that can mimic human gastric cardia cancer.

61.7 Conclusions

Research on *H. pylori* animal models is still underway, focusing on the mouse species considered best suited for *H. pylori* infection, especially a variety of transgenic or knockout mice. Despite various limitations as described above, such animal models greatly contribute to understanding the pathophysiology of human *H. pylori* infection. These studies are expected to make their due contributions to developing future vaccine, overcoming antibiotic resistance, and preventing cancer through carcinogenesis research. As such, research on animal models should be continued for the prevention of various *H. pylori*-associated gastric diseases.

References

1. Lee A. Animal models of *Helicobacter* infection. Mol Med Today. 1999;5:500–1.
2. Eaton KA. Animal models of *Helicobacter* gastritis. Curr Top Microbiol Immunol. 1999;241:123–54.
3. Lee A. Animal models for host-pathogen interaction studies. Br Med Bull. 1998;54:163–73.
4. Nedrud JG. Animal models for gastric Helicobacter immunology and vaccine studies. FEMS Immunol Med Microbiol. 1999;24:243–50.
5. O'Rourke JL, Lee A. Animal models of *Helicobacter pylori* infection and disease. Microbes Infect. 2003;5:741–8.
6. Fox JG, Cabot EB, Taylor NS, Laraway R. Gastric colonization by *campylobacter pylori* subsp. mustelae in ferrets. Infect Immun. 1988;56:2994–6.
7. Fox JG, Otto G, Murphy JC, Taylor NS, Lee A. Gastric colonization of the ferret with *Helicobacter* species: natural and experimental infections. Rev Infect Dis. 1991;13(Suppl 8):S671–80.
8. Kim YB, Hahm KB, Lee KM, Han SU, Kim MW. Animal models of *Helicobacter pyori* infections. Korean J Gastroenterol. 2001;37:399–405.

9. Shimizu N, Inada K, Nakanishi H, Tsukamoto T, Ikehara Y, Kaminishi M, et al. *Helicobacter pylori* infection enhances glandular stomach carcinogenesis in Mongolian gerbils treated with chemical carcinogens. Carcinogenesis. 1999;20:669–76.
10. Sugiyama A, Maruta F, Ikeno T, Ishida K, Kawasaki S, Katsuyama T, et al. *Helicobacter pylori* infection enhances *N*-methyl-*N*-nitrosourea-induced stomach carcinogenesis in the Mongolian gerbil. Cancer Res. 1998;58:2067–9.
11. Lee A, O'Rourke J, De Ungria MC, Robertson B, Daskalopoulos G, Dixon MF. A standardized mouse model of *Helicobacter pylori* infection: introducing the Sydney strain. Gastroenterology. 1997;112:1386–97.
12. Dey TK, Karmakar BC, Sarkar A, Paul S, Mukhopadhyay AK. A mouse model of *Helicobacter pylori* infection. Methods Mol Biol. 2021;2283:131–51.
13. Wang X, Willén R, Svensson M, Ljungh A, Wadström T. Two-year follow-up of *Helicobacter pylori* infection in C57BL/6 and Balb/cA mice. APMIS. 2003;111:514–22.
14. Takata T, El-Omar E, Camorlinga M, Thompson SA, Minohara Y, Ernst PB, et al. *Helicobacter pylori* does not require Lewis X or Lewis Y expression to colonize C3H/HeJ mice. Infect Immun. 2002;70:3073–9.
15. Ferrero RL, Thiberge JM, Huerre M, Labigne A. Immune responses of specific-pathogen-free mice to chronic *Helicobacter pylori* (strain SS1) infection. Infect Immun. 1998;66:1349–55.
16. Garhart CA, Redline RW, Nedrud JG, Czinn SJ. Clearance of *Helicobacter pylori* infection and resolution of postimmunization gastritis in a kinetic study of prophylactically immunized mice. Infect Immun. 2002;70:3529–38.
17. van Doorn NE, Namavar F, Sparrius M, Stoof J, van Rees EP, van Doorn LJ, et al. *Helicobacter pylori*-associated gastritis in mice is host and strain specific. Infect Immun. 1999;67:3040–6.
18. Kodama M, Murakami K, Nishizono A, Fujioka T. Animal models for the study of Helicobacter-induced gastric carcinoma. J Infect Chemother. 2004;10:316–25.
19. Pritchard DM, Przemeck SM. Review article: how useful are the rodent animal models of gastric adenocarcinoma? Aliment Pharmacol Ther. 2004;19:841–59.
20. Shomer NH, Dangler CA, Whary MT, Fox JG. Experimental *Helicobacter pylori* infection induces antral gastritis and gastric mucosa-associated lymphoid tissue in Guinea pigs. Infect Immun. 1998;66:2614–8.
21. Rijpkema SG, Durrani Z, Beavan G, Gibson JR, Luck J, Owen RJ, et al. Analysis of host responses of Guinea pigs during *Helicobacter pylori* infection. FEMS Immunol Med Microbiol. 2001;30:151–6.
22. Ansari S, Yamaoka Y. Animal models and *Helicobacter pylori* infection. J Clin Med. 2022;11:3141.
23. Hayakawa Y, Fox JG, Gonda T, Worthley DL, Muthupalani S, Wang TC. Mouse models of gastric cancer. Cancers (Basel). 2013;5:92–130.
24. Lee A, Fox JG, Otto G, Murphy J. A small animal model of human *Helicobacter pylori* active chronic gastritis. Gastroenterology. 1990;99:1315–23.
25. Mohammadi M, Czinn S, Redline R, Nedrud J. *Helicobacter*-specific cell-mediated immune responses display a predominant Th1 phenotype and promote a delayed-type hypersensitivity response in the stomachs of mice. J Immunol. 1996;156:4729–38.
26. Sakagami T, Dixon M, O'Rourke J, Howlett R, Alderuccio F, Vella J, et al. Atrophic gastric changes in both *Helicobacter felis* and *Helicobacter pylori* infected mice are host dependent and separate from antral gastritis. Gut. 1996;39:639–48.
27. Wang TC, Goldenring JR, Dangler C, Ito S, Mueller A, Jeon WK, et al. Mice lacking secretory phospholipase A2 show altered apoptosis and differentiation with *Helicobacter felis* infection. Gastroenterology. 1998;114:675–89.
28. Fox JG, Sheppard BJ, Dangler CA, Whary MT, Ihrig M, Wang TC. Germ-line p53-targeted disruption inhibits *Helicobacter*-induced premalignant lesions and invasive gastric carcinoma through down-regulation of Th1 proinflammatory responses. Cancer Res. 2002;62:696–702.
29. McCracken VJ, Martin SM, Lorenz RG. The *Helicobacter felis* model of adoptive transfer gastritis. Immunol Res. 2005;33:183–94.
30. Crabtree JE, Ferrero RL, Kusters JG. The mouse colonizing *Helicobacter pylori* strain SS1 may lack a functional cag pathogenicity Island. Helicobacter. 2002;7:139–40. author reply 40-1
31. Philpott DJ, Belaid D, Troubadour P, Thiberge JM, Tankovic J, Labigne A, et al. Reduced activation of inflammatory responses in host cells by mouse-adapted *Helicobacter pylory* isolates. Cell Microbiol. 2002;4:285–96.
32. Arnold IC, Lee JY, Amieva MR, Roers A, Flavell RA, Sparwasser T, et al. Tolerance rather than immunity protects from *Helicobacter pylori*-induced gastric preneoplasia. Gastroenterology. 2011;140:199–209.
33. Thompson LJ, Danon SJ, Wilson JE, O'Rourke JL, Salama NR, Falkow S, et al. Chronic *Helicobacter pylori* infection with Sydney strain 1 and a newly identified mouse-adapted strain (Sydney strain 2000) in C57BL/6 and BALB/c mice. Infect Immun. 2004;72:4668–79.
34. Hirayama F, Takagi S, Yokoyama Y, Iwao E, Ikeda Y. Establishment of gastric *Helicobacter pylori* infection in Mongolian gerbils. J Gastroenterol. 1996;31(Suppl 9):24–8.
35. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney system. International workshop on the histopathology of gastritis, Houston 1994. Am J Surg Pathol. 1996;20:1161–81.

36. Cai X, Carlson J, Stoicov C, Li H, Wang TC, Houghton J. *Helicobacter felis* eradication restores normal architecture and inhibits gastric cancer progression in C57BL/6 mice. Gastroenterology. 2005;128:1937–52.
37. Rogers AB, Fox JG. Inflammation and Cancer. I. Rodent models of infectious gastrointestinal and liver cancer. Am J Physiol Gastrointest Liver Physiol. 2004;286:G361–6.
38. Kim DH, Kim SW, Song YJ, Oh TY, Han SU, Kim YB, et al. Long-term evaluation of mice model infected with *Helicobacter pylori*: focus on gastric pathology including gastric cancer. Aliment Pharmacol Ther. 2003;18(Suppl 1):14–23.
39. Lee JY, Kim N, Nam RH, Choi YJ, Seo JH, Lee HS, et al. No correlation of inflammation with colonization of *Helicobacter pylori* in the stomach of mice fed high-salt diet. J Cancer Prev. 2014;19:144–51.
40. Lee JY, Kim N, Choi YJ, Nam RH, Choi YJ, Kwon YH, et al. Histologic findings and inflammatory reactions after long-term colonization of *Helicobacter felis* in C57BL/6 mice. J Cancer Prev. 2014;19:224–30.
41. Enno A, O'Rourke JL, Howlett CR, Jack A, Dixon MF, Lee A. MALToma-like lesions in the murine gastric mucosa after long-term infection with *Helicobacter felis*. A mouse model of *Helicobacter pylori*-induced gastric lymphoma. Am J Pathol. 1995;147:217–22.
42. Roth KA, Kapadia SB, Martin SM, Lorenz RG. Cellular immune responses are essential for the development of *Helicobacter felis*-associated gastric pathology. J Immunol. 1999;163:1490–7.
43. O'Keeffe J, Moran AP. Conventional, regulatory, and unconventional T cells in the immunologic response to *Helicobacter pylori*. Helicobacter. 2008;13:1–19.
44. Han JP, Hong SJ. Immune response to *Helicobacter pylori* infection. Korean J Helicobacter Up Gastrointest Res. 2013;13:220–3.
45. Zhang S, Moss SF. Rodent models of *Helicobacter* infection, inflammation, and disease. Methods Mol Biol. 2012;921:89–98.
46. Sawai N, Kita M, Kodama T, Tanahashi T, Yamaoka Y, Tagawa Y, et al. Role of gamma interferon in *Helicobacter pylori*-induced gastric inflammatory responses in a mouse model. Infect Immun. 1999;67:279–85.
47. Ohana M, Okazaki K, Oshima C, Andra's D, Nishi T, Uchida K, et al. A critical role for IL-7R signaling in the development of *Helicobacter felis*-induced gastritis in mice. Gastroenterology. 2001;121:329–36.
48. Berg DJ, Lynch NA, Lynch RG, Lauricella DM. Rapid development of severe hyperplastic gastritis with gastric epithelial dedifferentiation in *Helicobacter felis*-infected IL-10(−/−) mice. Am J Pathol. 1998;152:1377–86.
49. Danon SJ, O'Rourke JL, Moss ND, Lee A. The importance of local acid production in the distribution of *Helicobacter felis* in the mouse stomach. Gastroenterology. 1995;108:1386–95.
50. Ottlecz A, Romero JJ, Lichtenberger LM. *Helicobacter* infection and phospholipase A2 enzymes: effect of *Helicobacter felis*-infection on the expression and activity of sPLA2 enzymes in mouse stomach. Mol Cell Biochem. 2001;221:71–7.
51. Tu SP, Quante M, Bhagat G, Takaishi S, Cui G, Yang XD, et al. IFN-γ inhibits gastric carcinogenesis by inducing epithelial cell autophagy and T-cell apoptosis. Cancer Res. 2011;71:4247–59.
52. Smythies LE, Waites KB, Lindsey JR, Harris PR, Ghiara P, Smith PD. *Helicobacter pylori*-induced mucosal inflammation is Th1 mediated and exacerbated in IL-4, but not IFN-gamma, gene-deficient mice. J Immunol. 2000;165:1022–9.
53. Sayi A, Kohler E, Toller IM, Flavell RA, Müller W, Roers A, et al. TLR-2-activated B cells suppress *Helicobacter*-induced preneoplastic gastric immunopathology by inducing T regulatory-1 cells. J Immunol. 2011;186:878–90.
54. Sayi A, Kohler E, Hitzler I, Arnold I, Schwendener R, Rehrauer H, et al. The CD4+ T cell-mediated IFN-gamma response to *Helicobacter* infection is essential for clearance and determines gastric cancer risk. J Immunol. 2009;182:7085–101.
55. Ismail HF, Fick P, Zhang J, Lynch RG, Berg DJ. Depletion of neutrophils in IL-10(−/−) mice delays clearance of gastric *Helicobacter* infection and decreases the Th1 immune response to *Helicobacter*. J Immunol. 2003;170:3782–9.
56. Eaton KA, Mefford M, Thevenot T. The role of T cell subsets and cytokines in the pathogenesis of *Helicobacter pylori* gastritis in mice. J Immunol. 2001;166:7456–61.
57. Wang TC, Dangler CA, Chen D, Goldenring JR, Koh T, Raychowdhury R, et al. Synergistic interaction between hypergastrinemia and *Helicobacter* infection in a mouse model of gastric cancer. Gastroenterology. 2000;118:36–47.
58. Hasegawa S, Nishikawa S, Miura T, Saito Y, Madarame H, Sekikawa K, et al. Tumor necrosis factor-alpha is required for gastritis induced by *Helicobacter felis* infection in mice. Microb Pathog. 2004;37:119–24.
59. Yamamoto T, Kita M, Ohno T, Iwakura Y, Sekikawa K, Imanishi J. Role of tumor necrosis factor-alpha and interferon-gamma in *Helicobacter pylori* infection. Microbiol Immunol. 2004;48:647–54.
60. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature. 2000;404:398–402.
61. Tu S, Bhagat G, Cui G, Takaishi S, Kurt-Jones EA, Rickman B, et al. Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. Cancer Cell. 2008;14:408–19.
62. Houghton JM, Bloch LM, Goldstein M, Von Hagen S, Korah RM. In vivo disruption of the fas pathway abrogates gastric growth alterations sec-

ondary to *Helicobacter* infection. J Infect Dis. 2000;182:856–64.

63. Cai X, Stoicov C, Li H, Carlson J, Whary M, Fox JG, et al. Overcoming Fas-mediated apoptosis accelerates *Helicobacter*-induced gastric cancer in mice. Cancer Res. 2005;65:10912–20.
64. Chu IM, Hengst L, Slingerland JM. The Cdk inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy. Nat Rev Cancer. 2008;8:253–67.
65. Fero ML, Randel E, Gurley KE, Roberts JM, Kemp CJ. The murine gene p27Kip1 is haplo-insufficient for tumour suppression. Nature. 1998;396:177–80.
66. Kuzushita N, Rogers AB, Monti NA, Whary MT, Park MJ, Aswad BI, et al. p27kip1 deficiency confers susceptibility to gastric carcinogenesis in *Helicobacter pylori*-infected mice. Gastroenterology. 2005;129:1544–56.
67. Ohnishi N, Yuasa H, Tanaka S, Sawa H, Miura M, Matsui A, et al. Transgenic expression of *Helicobacter pylori* CagA induces gastrointestinal and hematopoietic neoplasms in mouse. Proc Natl Acad Sci U S A. 2008;105:1003–8.
68. Lemke LB, Ge Z, Whary MT, Feng Y, Rogers AB, Muthupalani S, et al. Concurrent *Helicobacter bilis* infection in C57BL/6 mice attenuates proinflammatory *H. pylori*-induced gastric pathology. Infect Immun. 2009;77:2147–58.
69. Ge Z, Feng Y, Muthupalani S, Eurell LL, Taylor NS, Whary MT, et al. Coinfection with enterohepatic *Helicobacter* species can ameliorate or promote *Helicobacter pylori*-induced gastric pathology in C57BL/6 mice. Infect Immun. 2011;79:3861–71.
70. Ferrero RL, Wilson JE, Sutton P. Mouse models of *Helicobacter*-induced gastric cancer: use of cocarcinogens. Methods Mol Biol. 2012;921:157–73.
71. Rolig AS, Shanks J, Carter JE, Ottemann KM. *Helicobacter pylori* requires TlpD-driven chemotaxis to proliferate in the antrum. Infect Immun. 2012;80:3713–20.
72. Koike K, Hinrichs SH, Isselbacher KJ, Jay G. Transgenic mouse model for human gastric carcinoma. Proc Natl Acad Sci U S A. 1989;86:5615–9.
73. Danon SJ, Eaton KA. The role of gastric *Helicobacter* and *N*-methyl-*N*'-nitro- *N*-nitrosoguanidine in carcinogenesis of mice. Helicobacter. 1998;3:260–8.

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